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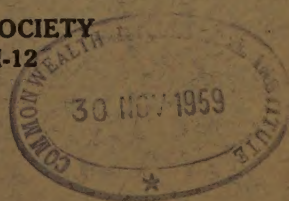
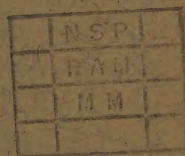
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## PRESIDENTIAL ADDRESS

### SOME DISEASES OF SUGARCANE REPORTED FROM INDIA IN RECENT YEARS\*

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Recent years have seen increasing attention of agriculturists being focussed on sugarcane, not only as an important cash crop but also a possible dollar earner for India. Every possible stress is now being laid by the Government to increase the yields of sugarcane from the existing cane acreage in the country, which has shown that it is not only a possibility but rather a certainty. The role of sugarcane diseases has long been realized in this respect. Intensive researches at the various Central Institutes and State Sugarcane Research Stations for devising ways and means to control some of the well established major diseases like Red Rot, Smut, Wilt and Mosaic, have yielded encouraging results. Intensive and systematic investigations on sugarcane diseases have brought to light some fresh problems as well, which, if present in the past, might have been overlooked, not being of any major consequence. These comprise maladies due to viruses, fungi, bacteria and flowering parasites apart from some of yet unknown origin. Some of these diseases which have been reported in recent years from India, or have assumed major importance, are briefly discussed.

**Mosaic.** Mosaic disease of sugarcane was the cause of great deal of apprehension when its occurrence in India was first discovered in 1921, owing to the fact that the disease was known to have caused considerable havoc in several cane-growing countries of the world. It was found to be present in a number of cane tracts of the country. Comprehensive studies at the I.A.R.I., however, showed that even a 100% mosaic affected crop resulted in a reduction in yield of only about 10% and that the juice quality remained unimpaired (Chona, 1944). These findings have naturally given a very much relegated position to the mosaic disease. It is, however, a source of potential danger, as it is possible that a new and more virulent strain other than the three known to be present in India, might arise in nature or may be inadvertently imported into the country, as has been the experience of Louisiana Sugar Industry, as also other cane growing countries of the world where mosaic is a serious disease. With this end in view, mosaic affected samples from different parts of the country are now being analysed for sugarcane mosaic virus strains. Indications of the existence of a new virulent strain causing severe mosaic and yellowing in Co. 527 in the Nellikuppam (South Arcot, Madras) area has been

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\*Presidential Address delivered at The Tenth Annual General Meeting of The Indian Phytopathological Society, January, 1958.

obtained. The occurrence of a more virulent strain, similarly, was also reported from Punjab, affecting Co. 313 (Singh, *et al*, 1957).

The Sugarcane Breeding Institute, Coimbatore, which is the main fountain-head of new sugarcane varieties of the country, is situated in a locality where there is active natural transmission of the disease and great care, therefore, has to be exercised in the selection of the seed-cane material when it is being distributed to the other Regional Sugarcane Research Stations and Central Institutes in the country, or sent abroad. In view of this, efforts are now being made to locate the seed nursery for multiplication of the seed-cane material for distribution, at a place named Talaparamba (West Coast) where there is no natural transmission of the disease.

**Grassy Shoot Disease.** The disease is characterized by the production of a large number of thin, small, spindly shoots, giving the plant a bushy appearance. The growth of the plant is arrested and the shoots remain very small and grassy. The disease is found chiefly in the cane variety Co. 419 in Bombay Deccan area (Albuquerque and Arakeri, 1957). Stray cases of this disease have been reported from the States of Madras and Andhra as well. In external symptoms, it has some resemblance with the *Sclerophthora* disease and the Australian Dwarf disease of sugarcane, but it is quite distinct from either. The disease is particularly pronounced in the ratoon crop (Vasudeva, 1956).

The disease is systemic and is perpetuated by the use of diseased seed-cuttings. The disease has been shown to be of virus origin, as it has been successfully transmitted by a species of aphid (*Longiungius sacchari*) as also by mechanical means. It has also been successfully transmitted to maize and *Sorghum vulgare*, which may thus act as collateral hosts. Its incidence and spread seems to be fortunately low and slow. The affected plants do not get killed. We have at the I.A.R.I. the first samples of the disease collected in 1949, which are still alive and are producing successively smaller and larger number of tillers.

It would be useful to consider now as an emergency measure the arrangements for replacement of Co. 419 with some other suitable and resistant cane variety in case it should become necessary. Hot water treatment (50°C. for 2 hours decided upon arbitrarily) of seed-cane material tried for the control of the disease has not proved to be successful and the cost of the treatment is estimated at Rs. 50/- per acre. Various other Temperature and Exposure ranges are being tested at Poona Sub-station to work out a suitable Hot Water Treatment.

Cases of Grassy Shoot Symptoms have been observed in some of the nucleus Seed-Cane material received from Sugarcane Breeding Institute in recent years at a few Stations and the possibility of the disease originating from the source should be checked up carefully, and necessary steps taken against any such dissemination of the disease.

**Albinism.** A new disease of sugarcane has recently been spotted in a few sugarcane tracts of India (Rafay and Singh, 1957). It is designated



by several names e.g. 'albinism' or 'chlorosis of leaves', etc. The affected plants may easily be spotted by disappearance of green colour from the leaves which are narrow, chlorotic and yellowish-white in colour. Such leaves have been observed to appear as early as the month of May in Muzaffarnagar on sugarcane variety Co. S. 245. With the advent of rains the symptoms become more noticeable.

Partial albinism has also been observed in ratoon crops of sugarcane varieties Co. 421 and Co. 527 at Rayagada (Orissa) where minute green patches were found to run longitudinally along the margins of an affected leaf while the rest of the leaf was devoid of chlorophyll. The chlorosis very often reaches the leaf-sheath as well.

Affected stalks are markedly stunted and thin. A pronounced tendency for tillering has also been observed in the affected plants. After the monsoon rains set in, numerous, white sickly tillers come out in a diseased stool, giving it a bushy appearance. Buds, on whatever few stalks are formed, germinate prematurely, resulting into long pale sprouts which is a characteristic feature of the disease.

Efforts to isolate any bacterium or fungus from the affected shoots have been unsuccessful. Application of glucose, potassium nitrate or molybdic acid have shown no improvement in the condition of an affected plant. Spraying of the diseased plants with ferrous sulphate has been observed to have no effect either. Work pertaining to the nature of this disease is now in progress at the I.A.R.I. as well as some other Sugarcane Research Stations.

**Spike Disease.** Spike disease of sugarcane has recently been recorded for the first time in Bihar (Sharma and Jha, 1957), where it is thought to be spreading. Spike disease is characterized by an abrupt and drastic reduction in the size of leaf lamina and leaf sheath of a mother shoot which had normal leaves to start with. There does not appear to be a corresponding decrease in the girth of the stalk and consequently sheaths are unable to encircle them and are pushed away. In cases of mild infection the disease is restricted only to a few internodes from where the stalk occasionally bends. A few buds within the affected portions may germinate to give rise to lateral shoots. In cases of severe infection, however, all the buds of the stalk might germinate. The cause of this disease is now being worked out at Pusa (Bihar) and is suspected to be of virus origin.

**Twisted Top.** Twisted top disease of sugarcane was first reported in 1954, from Maharashtra Sugar Factory Estate in Bombay - Deccan tract (Kharkar and Sabnis, 1954). This disease resembles the mechanical 'Tangle Top' in its symptoms but is probably different from that. The central spindle leaves and young leaves on the top are greatly entangled and twisted and give a braided appearance. The spindle of the plant affected with twisted top, soon ceases to grow. It is followed by sprouting of lateral buds into side shoots. In affected stools, the main as well as the side shoots have been observed to be markedly stunted and very thin. The setts (seed-cuttings) from the diseased canes have been found to give

rise to plants with normal healthy appearance. The true nature of the disease remains as yet undetermined.

**Red Stripe.** Though known for quite some time, Red-Stripe disease incidence had never been high as is evident from the reports received during recent years. This disease, caused by *Xanthomonas rubrilineas* (Lee *et al*) Dowson, has been reported from a number of other sugarcane growing countries of the world also where it is said to be associated with top-rot of the affected plants as well (Edgerton, 1955). Such an association, however, has not been observed in India.

During early stages of infection, elongated water - soaked streaks appear on the leaves. Later on, these streaks attain a dark red or maroon colour. These may appear on any part of the leaf-blade but are more common on the basal portions and are usually surrounded by pale or chlorotic zones. The stripes are long and narrow, in the beginning of the season but tend to run into each other forming broad bands later on. A few of these might extend to leaf-sheath also, where these are comparatively broader.

Red Stripe has always been considered a disease of minor importance in India. Occasionally it occurs in an epidemic form. Two such epidemics were observed at Kalai Farm (near Aligarh) and Risalewala (Lyallpur) affecting chiefly Co. 312 cane variety, years ago (1938, 1939). Recently, however, heavy incidence (upto 60%) was reported in some plots located in Phalton Sugar Factory Estate in Bombay-Deccan. (Chona, 1956).

Little work has been done on this disease in India, so far. It has, however, been observed that sugarcane varieties differ in their behaviour towards this disease and it would be desirable, therefore, to release only those varieties which are resistant to Red Stripe in areas where the disease is prevalent.

Some difficulty has been experienced in transmitting the disease under Delhi conditions with the local isolates. The taxonomy of the causal organism requires careful re-investigation.

**Rust.** Sugarcane rust, caused by *Puccinia kuehnii* (Krueg.) Butler, is assuming increasingly greater importance since its first reported occurrence in India in 1950, in a localised epidemic form (Chona and Munjal, 1950). Although the rust-affected specimens of sugarcane were deposited in Herb. Crypt. Ind. Orient. I.A.R.I., New Delhi, as far back as 1918, the disease was considered to be of rare occurrence till 1950, when it appeared in a serious form in Ahmednagar district of Bombay (Patel, *et al*, 1950), affecting a very promising commercial and popular cane variety, Co. 475. The rust has subsequently been reported to occur on Co. 678, Co. 718, Co. 785, Co. 787, Co. 792, Co. 915, Co. 990, Co. 992, Co. 993, Co. 1019, Co. 1022, Co. 1025, Co. 1106, Co. 1141, and Co. S. 510. On account of the repeated recurrence of this disease each year on Co. 475, the Bombay Department of Agriculture have now, on the advice of the Indian Central



Sugarcane Committee, withdrawn this variety (Co. 475) from general cultivation, inspite of its excellent performance. There is, however, little data available about the magnitude of losses due to sugarcane rust, as yet.

Upto 1955-56, the disease in an epidemic form, was considered to be confined to South India cane tract only and appeared late in the season during the cooler months—October to January. Sudden outbreak of the rust in an epidemic form was observed during February, 1956, in Northern India at Gola-Gokaran-nath near foot-hills of Nepal, in U.P., affecting a recently released cane variety, Co. S. 510, which occupies an area of over 5,000 acres. The disease first appeared on the October-planted crop and later had spread to the new young, February-sown crop and ratoon crop as well. The attack was so severe that the badly affected fields gave the appearance as if the crop had been burnt. The affected crop, however, recovered remarkably well by the following October.

The disease manifests itself in the form of numerous small elliptic, brownish to tawny coloured pustules. With the formation of teleutospores, the pustules attain a darker colour. As a result of infection, leaves dry up giving a set-back to the crop in case of severe infection. The uredospores of the fungus germinate freely in water within 12 hours over a wide range of temperature, from 16 to 29°C, the optimum being 20–25°C (Chona, *et al*, 1950; Vasudeva, 1956). Teleutospores too, have been observed to germinate, though sparingly, giving rise to a promycelium and four sporidia (Sahni, 1957).

Rust infection has been observed on certain plant species closely related to sugarcane, viz. *Saccharum sara*, *S. narenga*, *S. arundinaceum*, *S. spontaneum*, *Erianthus fulvus* and *Sclerostachya fusca*. The rust occurring on some of these hosts closely resembles the one occurring on sugarcane. Preliminary tests conducted at the I.A.R.I. have shown that *S. spontaneum* and *E. fulvus* rusts are capable of infecting sugarcane (Sahni 1957). It might, however, be mentioned that several *S. spontaneum* and *Erianthus* variants are found affected at S.B.I., Coimbatore, but the rust has not so far infected any of the very large number of cane varieties, including susceptible varieties, like Co. 457, growing in close proximity at the Station. A similar situation has been reported from Sugarcane Research Station, Pusa (Bihar) also.

For further investigation regarding the life history of the rust, Physiologic Forms, Resistant Varieties, etc. a special Co-ordinated Scheme has recently been sanctioned by the I.C.S.C to be worked out at the I.A.R.I. and the State Sugarcane Research Stations concerned. The taxonomy of the Rust also needs careful investigation.

**Red Rot of Leaf Sheath.** Red rot of leaf sheath, caused by *Sclerotium rolfsii*, has been reported to have been responsible for extensive damage in some localities in Bombay-Deccan area, during the 1954-55 season (Albuquerque and Arakeri, 1957). The disease manifests itself

in the form of blood-red discolouration of the affected leaf sheaths which is visible on both the surfaces. During the periods of high humidity and comparatively low temperature profuse mycelial growth of the causal fungus may be observed between the leaf-sheath and the stalk (Albuquerque, 1957). In cases of severe infection small scars may appear on the rind apart from occasional eye-bud rot. Though the reddening does not extend to the leaf blade, the adverse effect of the disease is quite marked on the leaf blades. Starting from a slight pallor of the base of the leaf blade, the leaves gradually dry up and die. Almost all the shoots of a diseased stool are usually thin, stunted and with many short internodes. It has been observed that the incidence of the disease is directly related to high humidity and decreases with the rise in temperature.

**Yellow Leaf Spot.** *Helminthosporium*, *Leptosphaeria* and *Cercospora* spots on sugarcane leaves are of common occurrence in India. Two species of *Cercospora* are usually found attacking sugarcane leaves, viz., *C. longipes* and *C. kopkei*. The latter which causes yellow leaf disease has been responsible for serious outbreaks of this disease in Java and Queensland, in the past. In our country, yellow leaf spot disease has always been of minor importance and reported only from Assam. Recently, however, during the last few years it has been reported to assume an important role in South India, chiefly affecting Co. 419, the main commercial cane variety of the tract. The disease has been prevalent in the coastal areas.

Early stages of attack of this fungus are characterised by the appearance of pallid, yellow spots on the leaves, which gradually grow larger and finally coalesce forming irregular patches with a shade of red. In cases of severe infection, whole leaf may turn reddish-brown at first and finally straw-coloured on drying up. Investigations are now being conducted at the Sugarcane Research Stations concerned with regard to assessment of losses and measures for control in case this disease assumes a serious proportion and becomes a major problem of Sugarcane Industry in the near future. Varietal resistance trials have shown sugarcane variety Co. 449 to be resistant to this disease in South Kanara.

**Leptosphaeria Leaf Spot.** *Leptosphaeria* leaf-spot caused by *Leptosphaeria sacchari* Butler, and considered to be of only minor importance in the past in India, has been reported to cause considerable damage to certain cane varieties at Talaparamba, and in certain coastal areas of Andhra State. The disease was claimed to be so serious by the cane growers in Andhra during the 1955-56 season, as to interfere with jaggery setting. However, this view is not being supported by the local Sugarcane Research Station, Annakapalle.

**Top Rot.** The disease is caused by *Fusarium moniliforme* Scheld. and is similar to "Pokkah Boeng" of Java but is not of much economic importance under Northern India conditions. The disease is omnipresent during the rainy season affecting almost all the cane varieties to a greater or lesser extent but the affected plants recover completely, later in the season. \* A severe type of Top Rot infection has, however, been observed in Nellikuppam (S. Arcot, Madras) area (Chona, 1956). Though the normal



growth is resumed later in the season by the affected plants, the point of infection remains a weak spot and often the cane stalk breaks off at this point during the heavy winds which are quite common in that area and thus cause considerable economic loss.

**Seedling Blight.** In raising the sugarcane seedlings from true-seed, for breeding purposes at Coimbatore, a serious disease that usually takes a heavy toll is "seedling blight". Isolations from affected seedlings have revealed the presence of usually three species of *Pythium* (*P. graminicolum*, *P. aphanidermatum* and *P. catenulatum*) and occasionally *Rhizoctonia solani* (Srinivasan and Chenulu, 1957). Pathogenicity trials have shown that each one of these organisms except *P. catenulatum*, is capable of causing severe root-rot of seedlings. This disease has been successfully controlled by heat sterilization of soil or by post-emergence drenching of the growing seedlings with chemicals like 1% formalin and Cheshunt compound. Treatment with Cheshunt compound has been claimed to be the best (Srinivasan and Chenulu, 1957).

**Sugary Disease.** The occurrence of Ergot sclerotia on sugarcane "arrows" was reported in 1943, from Mysore but the conidial stage of the fungus had not been described (Thirumalachar, 1943). During 1954, arrows of one of the clones of *Saccharum spontaneum* hybrids in the nobilization series at the Sugarcane Breeding Institute, Coimbatore, were reported to be affected with sugary disease. From most of the spikelets a sweet, straw-coloured fluid was found oozing out emitting a strong odour characteristic of ergot infections (Srinivasan and Chenulu, 1956). The "honey dew" revealed the presence of numerous conidia of *Sphacelia*. In the absence of sclerotial formation it was not possible to identify the species of *Claviceps* involved. The fungus may possibly be identical to the one reported from Mysore producing Ergot disease of Sugarcane.

**Phanerogamic Parasites.** *Striga* is a common phanerogamic root-parasite of sugarcane in several localities in the various cane-tracts of the country. Three different species of *Striga* are known to attack sugarcane, namely, *Striga lutea* Lour, *Striga densiflora* Benth, and *Striga euphrasoides* Benth. Application of 2-4, D (Methaxon, Agraxon, etc.) is known to successfully control *Striga* infestation. Another flowering plant, *Sopubia delphenifolia* has recently been reported to be parasitising sugarcane from Bihar (Sharma and Trivedi, 1957). Apart from sugarcane it has been found to be capable of parasitising a large number of other plant species also.

#### CONCLUSION

The incidence of these diseases has been comparatively limited upto this time, and none of these have, fortunately, as yet, caused wide-spread and extensive losses as Red Rot, in our country. Only sporadic reports have been received about these from time to time from different sugarcane growing areas of India. It is, however, felt very necessary that these are carefully watched and studied systematically and, as an emergency measure, the arrangements should be envisaged for the replacement of varieties which are susceptible to these, in case it should become necessary. This

is obviously the duty of all the well-wishers of Sugar Industry in the country particularly the Sugarcane Pathologists and generally of the Members of the Indian Phytopathological Society, who, I feel sure, would rise to the occasion and do the needful towards the study of these so-called minor diseases of an important economic crop on which is dependent the second largest Industry of the country.

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## THE MYXOMYCETES OF THE MUSSOORIE HILLS - IX

K. S. THIND AND M. S. MANOCHA

(Accepted for publication December 1, 1957)

The first eight contributions (listed under references) by the senior author and his students describe 55 Myxomycetous fungi including three new species. This ninth paper on the series deals with 10 more species of which 9 are new records for the Mussoorie Hills while 5 are new records for India.

The Classification of Martin, 1949, has been followed throughout this study, although monographs of Lister and Lister, 1925, and Macbride and Martin, 1934, were freely consulted.

The numbers of the species are the serial numbers of the Myxomycetous flora of the Mussoorie Hills.

Type collections have been deposited in the Herbarium of the Punjab University and Herbarium Crypt. Ind. Orient. New Delhi;

The authors are deeply indebted to Prof. P. N. Mehra, Head of the Panjab University Botany Department, for encouragement and providing facilities.

### 56. *Leocarpus fragilis* (Dicks.) Rost.

*Fructifications* sporangiate; sporangia densely gregarious forming large clusters up to 4.5 cm., stipitate, yellowish brown to chestnut brown to reddish brown, shining, obovate or pyriform, 0.7 – 0.9 mm. wide and 1 – 1.5 mm. long; stipe short to long, very weak and hence the sporangia mostly lying flat on the substratum, simply an extension of the membranous hypothallus, thin, membranous or flattened, yellow or light yellow to cream coloured, 0.1 – 0.7 mm. long; hypothallus thin, membranous, confluent, whitish; peridium double; outer peridium shining, crustose or shell-like, smooth and glabrous, brittle, cartilaginous, calcareous within; inner peridium membranous, thin, hyaline; dehiscence irregular.

*Capillitium* duplex, one system composed of yellowish orange, strongly calcareous tubules, the latter being mostly elongate and branched irregularly; the other system composed of a dense network of hyaline, slender, flattened, non-calcareous, internodal threads, the latter being often expanded at the axils.

*Spores* black in a mass, violaceous brown under the microscope, globose, coarsely verrucose, 10.8 – 14  $\mu$  in diameter.



Text Fig. 1, A--C.



Text-Fig. 1. *Leocarpus fragilis* (Dicks.) Rost.. A. A cluster of sporangia with slender weak stipes, x 20. B. Duplex capillitium, x 400. C. Coarsely verrucose spores, x 1000.

Collected on bark of *Cedrus deodara*, Kanatal forest, Mussoorie, August 22, 1956, 234. On dead coniferous needles (of *Pinus* and *Cedrus*) and on dead wood, Kodia forest, August 21, 1956, 235.

This very beautiful and easily recognizable species seems to be quite common in Kanatal and Kodia forests but has not been observed in Mussoorie proper so far. Probably it grows at higher altitude under cooler conditions. The species is characterized by the gregarious habit, shining and pyriform sporangia, weaker stipes, double peridium, and duplex capillitium. It is not likely to be confused with other Myxomycetes.

#### 57. *Diderma spumarioides* (Fries) Fries

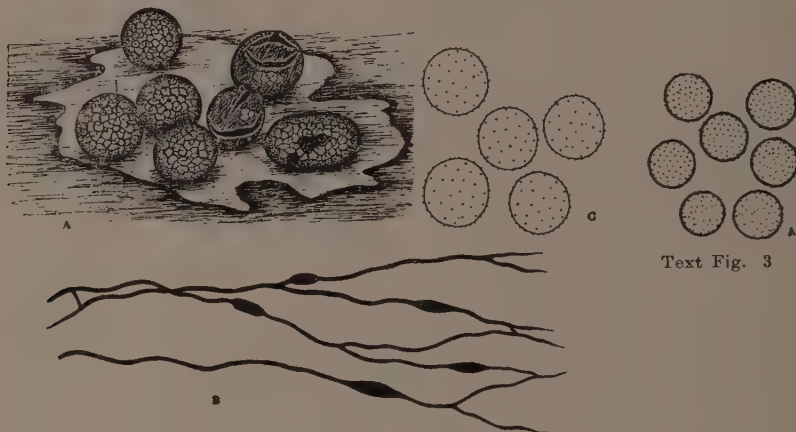
*Fructifications* sporangiate; sporangia densely gregarious to closely crowded, sessile, pulvinate, globose to sub-globose, sometimes elongated slightly so as to appear short, straight, plasmodiocarps, white areolate, 0.34–0.67 mm. in diameter, up to 0.85 mm. long in case of plasmodiocarpous types; peridium double, the two walls closely applied; outer peridium densely calcareous, white, fragile, areolate, calcareous matter composed of minute, spherical granules, closely adhering to the inner peridium; inner peridium membranous, ash-coloured; hypothallus profusely developed, confluent or common to a sporangial mass, white, calcareous, extending much beyond the sporangial cluster, the sporangia more or less embedded in it; dehiscence irregular.

*Columella* conspicuous, hemispherical or dome shaped, appearing like the raised base, light brown.

*Capillitium* well developed, violaceous brown, non-calcareous, composed of branching and anastomosing sparsely, thin, slender, flexuous threads with paler coloured extremities and marked by fusiform or elongated nodular thickening.

*Spores* black in a mass, violaceous brown under the microscope, globose, distinctly but rather sparsely verrucose,  $8.8 - 10.4 \mu$  in diameter.

Text-Fig. 2 A - C. & 3 A.



Text Fig. 2

Text-Fig. 2. *Diderma spumarioides* (Fries) Fries, A. Sporangia with areolate outer peridium,  $\times 20$ . B. Capillitium marked by fusiform nodular thickenings,  $\times 400$ . C. Sparsely but distinctly verrucose spores,  $\times 1000$ .

Text-Fig. 3. *Comatricha pulchella* (C. Bab.) Rost., A. Minutely verrucose spores,  $\times 1000$ .

Collected on living mosses, dead leaves of *Quercus incana*, and dead leaves of grasses, Pari Tibba, Mussoorie, September 1, 1956, 236.

This fungus from the Mussoorie hills represents rather a typical *Diderma Spumarioides* (Fries) Fries and is at once recognized by white globose sporangia crowded on a profusely developed hypothallus, outer heavily calcareous peridium closely adhering to the inner membranous peridium, distinctly but rather sparsely verrucose spores, and well developed capillitium.

#### 58. *Comatricha pulchella* (C. Bab.) Rost.

*Fructifications* sporangiate, total height 0.6 - 1.1 mm., gregarious, stipitate; sporangia ovate or ovato-oblong to short cylindric, apex rounded to acute or appearing mucronate in some cases, sometimes confluent, dark brown, 0.37 - 0.72 mm. long, 0.2 - 0.3 mm. wide; stipe black, short, shining, slender, even, 0.25 - 0.4 mm. long, less than one-half of the total height; hypothallus distinct, membranous, dark brown, circular; peridium evanescent; dehiscence irregular.

*Columella* prominent, simply a prolongation of the stipe, black, gradually tapering upward, merging into the capillitium just below the apex.



*Capillitium* dense, arising all over the columella, composed of flexuous violaceous brown threads which anastomose freely, forming a *Stemonitis*-like net, meshes wide, up to  $30\ \mu$  in diameter, capillitial threads looped at the surface, free ends few, surface net in the sense of *Stemonitis* lacking.

*Spores* brown in a mass, light violaceous brown under the microscope, globose to sub-globose to ovoid, minutely and uniformly verrucose,  $6.8 - 8.4\ \mu$  in diameter.

## Plate I



*Comatricha pulchella* (C. Bab.) Rost.

Collected on dead twigs, Brewery Road, Mussoorie, August, 14, 1956, 237.

This collection undoubtedly belongs to *Comatricha pulchella* (C. Bab.) Rost. and is characterized by the short, ovate to short cylindric sporangia, flexuous capillitial threads, looped at the ends, freely anastomosing to form a wide meshed net, and with a few free ends, minutely verrucose spores. This Mussoorie collection resembles variety *fusca* Lister in the purplish brown, more rigid capillitium, and lighter coloured spores.

59. *Perichaena chrysosperma* (Currey) Lister

*Fructifications* plasmodiocarpous, with a few sporangial types; plasmodiocarps scattered or gregarious, dark brown or dark chestnut brown coloured to almost black, short, straight or bent to curved, "U" shaped, or annular, 0.28 - 0.5 mm. in diameter, reduced to spherical sporangial bodies in a few cases; hypothallus none; peridium single, thick, darker on the outside, lighter coloured on the inside, marked on the outside by dark coloured granular deposits, marked on the inside by very fine dark coloured irregular lines; dehiscence irregular.

*Capillitium* well developed, yellow, composed of long, slender, highly convoluted, sparingly branched threads,  $2.4 - 3.2 \mu$  in diameter, not uniform in thickness, profusely and prominently spinulose, spines short to long, sharp-pointed, straight or bent, slender, smooth to rough, up to  $4 \mu$  long.

*Spores* yellow in a mass, light yellow under the microscope, globose, profusely verrucose, warts minute but very distinct, uniguttulate, guttule filling about three-fourth of the spore cavity,  $10 - 11.2 \mu$  in diameter.

Text-Fig. 4, A C.



Text-Fig. 4. *Perichaena chrysosperma* (Currey) Lister, A. Annular to curved plasmodiocarps,  $\times 20$ . B. Prominently spiny capillitial thread,  $\times 1000$ . C. Minutely verrucose spores,  $\times 1000$ .



Collected on dead and decaying leaves of *Agave sp.*, Adunca Bridge, Mussoorie, Sept. 8, 1956, 238.

This collection closely resembles *Perichaena chrysosperma* (Currey) Lister in all respects.

The peridium in the Mussoorie collection appears to be single except for the grannular deposit on the outside, which may be called outer peridium. The outer peridium is described as composed of grannular material (see Martin, monograph 1949).

#### 60. *Arcyria versicolor* Phill.

*Fructifications* sporangiate; sporangia densely crowded or heaped together in large clusters extending up to 3.5 cm., stipitate, ovato-cylindric to ovate, deep brown to dark brown, lower part shining bright coloured, iridescent above, 0.9 – 1.6 mm. long and 0.5 – 0.8 mm. wide; stipe short to long, slender, weak, strand-like, dark brown or concolorous with the sporangia, filled with spore-like cells, much bigger than the spores, longitudinally grooved, 0.5 – 1 mm. long, expanded above into the sporangium; hypothallus well developed, dark brown, mostly confluent into thin sheets; peridium persistent, single, membranous, shining, goblet-shaped below, iridescent above; dehiscence irregular, peridium rupturing at the top, whole of the upper iridescent part falling off but the lower, shining, goblet-shaped part remaining persistent as deep cup shaped, vasiform calyculus after spore discharge.

*Capillitium* well developed, only slowly and slightly expanded, free from the calyculus, reddish brown, i.e. concolorous with the spores, yellowish brown under the microscope, composed of convoluted threads forming a loose net work. Capillitial threads sparsely and elegantly branched, abundantly spinulose, also marked by faint reticulations or incomplete

Text-Fig. 5, A – C.



Text-Fig. 5. *Arcyria versicolor* Phill., A. Stipitate sporangia with clearly demarcated lower goblet-shaped persistent portion, x 20. B. Abundantly spinulose capillitial thread with faint spirals, x 1000. C. Faintly roughened spores, x 1000.

faint spirals, spines sharp pointed, up to  $3.2\ \mu$  long,  $4 - 5\ \mu$  in diameter, apices obtuse to acute, sometimes slightly enlarged and swollen.

*Spores* reddish brown in a mass, pallid (almost subhyaline) under the microscope, globose to ovoid, smooth, or very faintly roughened,  $7 - 8.8\ \mu$  in diameter.

Collected on dead wood, Kanatal Forest, September 8, 1955, 239.

This beautiful species is marked by closely clustered, ovato-cylindric sporangia, peridium forming vasiform calyculus after dehiscence, capillitium free from the calyculus and smooth spores. However, in Mussoorie collection the spore size is smaller for the species.

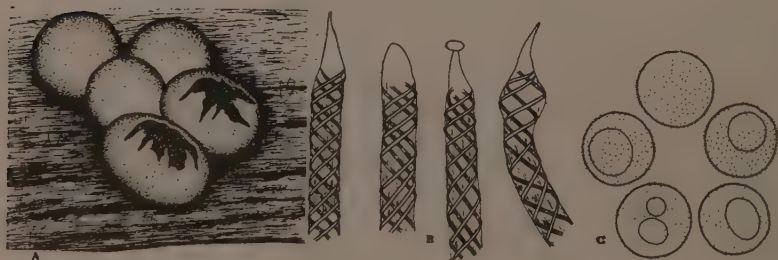
#### 61. *Trichia lutescens* Lister

*Fructifications* sporangiate: sporangia gregarious, densely crowded or in small clusters, sessile to short stipitate, globose or pulvinate, often becoming elongated due to mutual compression or even otherwise, yellow to yellowish brown to deep brown, shining,  $0.58 - 0.85$  mm. in diameter up to  $1.3$  mm. long in the case of elongated sporangia: stipe, when present, very short, inconspicuous to distinct, stout, dark brown, slightly rugulose, up to  $0.15$  mm. long: hypothallus small, dark brown: peridium single, thin, membranous, shining, iridescent, translucent, yellow, without granular deposits, embossed with the impressions of the spores: dehiscence irregular, lower part of the peridium remaining persistent.

*Columella* none.

*Capillitium* well developed, yellow, consisting of short to long, simple to rarely branched, highly convoluted (forming loops), free elaters,  $3.6\ \mu$  wide, apices acuminate to blunt to obtuse to knobbed, uniform in width, occasionally marked by conspicuous, small to large swellings, marked by  $3 - 5$  spiral bands, spiral bands mostly distinct, sometimes faint, regular to irregular making the elaters coarsely verrucose at several places.

Text - Fig. 6, A - C.



Text-Fig. 6. *Trichia lutescens* Lister. A. Sporangia,  $\times 20$ . B. Elaters with variable apices,  $\times 1000$ . C. Minutely but distinctly verrucose spores with  $1 - 2$  guttules,  $\times 1000$ .

*Spores* yellow in a mass, light yellow under the microscope, globose, profusely verrucose, warts minute, very distinct and uniformly distributed, 1 - 2 guttulate, 11 - 14  $\mu$  in diameter, mostly 12 - 13  $\mu$  in diameter.

Collected on dead wood, Kodia forests, Mussoorie, Sept. 12, 1955, 240. On dead and decaying wood, The Park, Mussoorie, August 25, 1955, 241.

This species is marked by shining yellow, sessile to short stipitate sporangia, translucent peridium and minutely verrucose, large spores. It is differentiated from the closely allied *Trichia contorta* (Ditmar) Rost. by its translucent peridium free from granular deposits but marked with the impressions of the contained spores. Both the Mussoorie collections fall well within *Trichia lutescens* Lister. The sporangia of the collection from the Park are more frequently stipitate and brighter yellow coloured than those of the collection from Kodia forest. In all other aspects the two resemble closely.

62. *Trichia pusilla* (Hedw.) G. W. Martin

*Frutifications* sporangiate; sporangia densely gregarious to crowded in clusters, stipitate, olivaceous brown or olivaceous yellow, shining, iridescent, turbinate or pyriform, 0.6 - 0.9 mm. broad, up to 1.2 mm. long, total height up to 1.8 mm.; stipe erect, brown, cylindrical, longitudinally grooved, 0.2 - 0.7 mm. long, filled with spore-like cells; hypothallus brown, well developed, membranous, often confluent; peridium single, thin, membranous, yellow, shining, iridescent, translucent; dehiscence irregular.

Text - Fig. 7, A - C.



Text-Fig. 7. *Trichia pusilla* (Hedw.) G. W. Martin. A. Sporangial cluster arising from a common hypothallus, x 20. B. Simple to branched elaters, sometimes marked by a large swelling, x 1000. C. Incompletely verrucose spores, x 1000.



*Capillitium* abundant, yellow or olivaceous yellow, composed of free elaters; elaters  $4.5 - 6 \mu$  thick in the centre, simple to branched, branches irregular and  $1 - 4$ , tapering gradually to long slender apices, marked by  $4 - 5$  spiral bands, sometimes swollen at one or two places at the origin of branches or otherwise, swollen parts sac-like and smooth or marked by  $4 - 5$  smooth spiral bands.

*Spores* olivaceous yellow in a mass, light yellow under the microscope, globose to subglobose, delicately and incompletely reticulate, meshes irregular, minutely verrucose in the remaining portion,  $10.4 - 13.2 \mu$  in diameter.

Collected on dead wood, The Park, Mussoorie, Sept. 7, 1956,  
242.

This fungus undoubtedly belongs to *Trichia pusilla* (Hedw.) G.W. Martin and is marked by olivaceous brown or olivaceous yellow, turbinate and stipitate sporangia, simple to branched elaters tapering to long, slender apices and incompletely reticulate spores with the balance minutely warted.

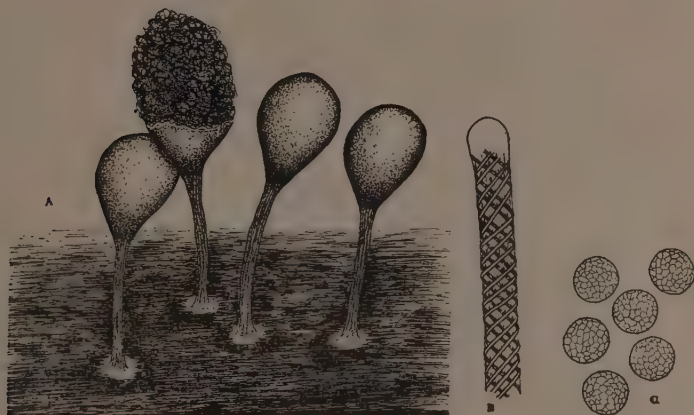
63. *Hemitrichia stipitata* (Masse) Macbr.

*Fructifications* sporangiate, total height up to 2.2 mm.; sporangia scattered to gregarious, stipitate, erect or bent, turbinate, golden yellow to yellowish brown, upto  $0.9 \times 0.7$  mm. when intact; stipe long, slender, uniform in thickness, erect to bent, not expanding above into the sporangium, ending abruptly below the calyculus, reddish brown, hollow, filled with spore-like cells, longitudinally grooved,  $0.8 - 1.3$  mm. long; hypothallus distinct, reddish brown, thin; peridium single, membranous, translucent, lower half longitudinally ridged and lighter coloured, upper half somewhat iridescent, minutely papillate on the inner side; dehiscence irregular, the upper half falling away but the lower half remaining persistent as a shallow petaloid calyculus.

*Capillitium* abundant, dense, elastic, considerably swollen after dehiscence, yellowish brown to the unaided eye, brown yellow under the microscope, forming a loose, wide-meshed net, with few free obtuse ends; capillitial threads  $4 - 6 \mu$  wide, marked by  $5 - 6$  regular spirals, smooth, sparingly branched, highly convoluted, attached below to the base of the calyculus.

*Spores* yellow in a mass, light yellow under the microscope, globose, very minutely verrucose and marked by very delicate, fine, reticulations observed only under oil immersion lens, appearing smooth under high power of the microscope,  $6.4 - 8.8 \mu$  in diameter, mostly  $6.8 - 7.6 \mu$  in diameter.

Text - Fig. 8, A - C.



Text-Fig. 8. *Hemitrichia stipitata* (Masse) Macbr., A. Long stipitate sporangia, with a persistent basal calyculus and an elastic capillitium shown in one after dehiscence,  $\times 20$ . B. An elater with obtuse apex,  $\times 1000$ . C. completely and delicately reticulate spores,  $\times 1000$ .

Collected on dead wood and alive mosses, Chakrata Toll, Mussoorie, August 1, 1956, 243. On dead wood Dhobi Ghat, Mussoorie, July 28, 1956, 244.

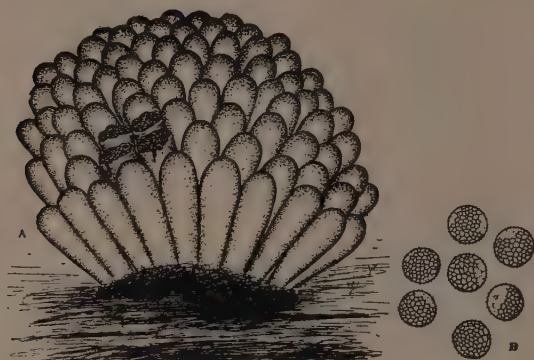
This species is quite common in the Mussoorie Hills and is marked by turbinate stalked sporangia, stalk ending abruptly below the petaloid calyculus, smooth and dense capillitium and minutely verrucose and faintly reticulate spores.

64. *Tubifera ferruginosa* (Batsch) J. F. Gmel.

*Fructifications* sporangiate, sporangia arranged in pseudo-aethaloid clusters up to 2.5 cm. broad; sporangia cylindric, slightly narrowing downward, wall entire but connate, free at the top, apex rounded, brown, up to 2 mm. long and up to 0.53 mm. wide; hypothallus prominent, well developed, spongy, white or pallid when covered with spores; peridium single, thin, membranous translucent, iridescent, persistent, papery and wrinkled after spore discharge; dehiscence apical, at first opening by a small pore, later on whole of the top breaks away irregularly, the lower peridium remaining persistent as an open tube; columella none; capillitium none.

*Spores* brown in a mass, light brown under the microscope globose, reticulate, reticulations present on three-fourth of the spore surface, the remaining one-fourth smooth or marked with broken ridges, (reticulations extending beyond the spore surface as sharp warts and hence appearing verrucose in a sectional view), 5.2 - 6.4  $\mu$  in diameter.

Text-Fig. 9, A - B.



Text-Fig. 9. *Tubifera ferruginosa* (Batsch.) J. F. Gmel., A. pseudo-aethaloid cluster of sporangia on a well-developed spongy hypothallus, x 10. B. Incompletely reticulate spores, x 1000.

Collected on dead stumps of *Quercus incana*, Dhenolti, Mussoorie, August 22, 1956, 245.

This collection undoubtedly belongs to *Tubifera ferruginosa* (Batsch) J. F. Gmel and is recognized by the large connate clusters of sporangia, spongy hypothallus, persistent peridium, and markedly reticulate spores. The measurements of the Mussoorie collection for fruit bodies and spores are smaller for the species.

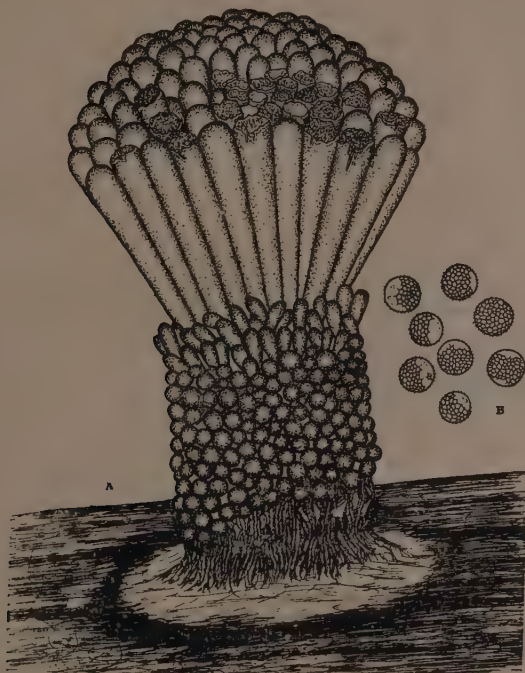
#### 65. *Tubifera microsperma* (Berk. & Curt.) G. W. Martin

*Fructifications* sporangiate, sporangia clustered into a pseudo-aethalium which is raised on a stalk-like hypothallus, pseudoaethalium up to 9 mm. tall and up to 1.8 cm. broad, the stalk like hypothallus often surrounded by shorter clusters of later formed smaller sporangia; sporangia cylindric, narrowing downward, apex rounded, brown, walls entire but connate, up to 4 mm. long and up to 0.42 mm. wide; hypothallus columnar stipe-like, spongy or sulcate, 3-5 mm. long, becoming surrounded by later developed small clusters of very small sporangia, hence becoming obscure except at the base; peridium single, thin, membranous, translucent, somewhat iridescent; dehiscence apical, peridium rupturing at the top and its lower part remaining persistent as an open long tube; columella none; capillitium or pseudocapillitium none.

*Spores* brown in a mass, light brown under the microscope, globose, reticulate, reticulations complete on three-fourth of the spore surface, the remaining one-fourth smooth or marked with broken ridges, reticulation uniform and extending beyond the spore surface as warts and hence spores appearing verrucose in a sectional view, 4.8 - 6  $\mu$  in diameter.



Text-Fig. 10 A - B.



Text-Fig. 10. *Tubifera microsperma* (Berk. & Curt.) G. W. Martin, A. Pseudo-aethaloid cluster of sporangia on a broad, stalk-like hypothallus,  $\times 10$ . B. Incompletely reticulate spores,  $\times 1000$ .

Collected on dead and decaying wood of *Quercus incana*, The Park, Mussoorie, July 30, 1956, 246.

This species is marked by stipe-like hypothallus and in this respect it differs from *Tubifera ferruginosa* (Batsch) J. F. Gmel.

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## PRELIMINARY NOTE ON AN ACTINOMYCIN PRODUCED BY A STREPTOMYCES SPECIES

M. J. THIRUMALACHAR AND D. GHOSH.

(Accepted for publication December 4, 1957)

A *Streptomyces* species isolated from Poona soil samples and found to produce an actinomycin type of antibiotic has been studied in detail. The organism grows readily on most of the media commonly employed for culturing actinomycetes. The colonies are fast growing at the optimum temperature of 28°C. The vegetative growth is composed of long branching filaments developing cinereous powdery mycelium. The sporophores are long, clustered, straight and dichotomously branched, the branches being upturned without producing any spirals. The colonies are non-chromogenic but producing an orange-yellow soluble pigment on most of the agar media. On Czapek's agar, growth is slow with powdery white aerial mycelium and very little yellow pigment production in the medium. On glucose asparagin agar, growth is colourless, with white powdery mycelium and faint yellow colour in the medium. On starch agar, there is slight hydrolysis with vigorous growth of the aerial mycelium and good production of yellow pigment in the medium. Nitrates are slightly reduced to nitrite. Litmus milk turns blue without evident coagulation. Following abundant spore production, the colonies turn pale olive-yellow and the spores are ovate-elliptic to spherical.

Comparative studies have indicated that the *Streptomyces* species under study should be classified under *S. chrysomallus* Lindenbein. *S. michiganensis* Corbaz et al (1956) is a chromogenic species. *S. chrysomallus* has been studied in detail by Brockmann et al (1949) for the production of the interesting antibiotic actinomycin-C group. Investigations by Ravina et al (1956) have shown the potential use of actinomycin-C due to its cytostatic action in the treatment of malignant tumors.

In preliminary screening tests on agar media, the antibiotic produced by the strain of *S. chrysomallus* under study, was found to inhibit a number of Gram positive bacteria including *Bacillus subtilis*, *B. mycoidea*, *Micrococcus pyogenes* var *aureus*, *Sarcina lutea* and the acid-fast organism *Mycobacterium phlei*. There was only trace or no activity against *Escherichia coli*. This has been confirmed by studying the antimicrobial spectra using pure crystalline antibiotic. 0.4 micrograms per ml. of the antibiotic completely inhibited the growth of *M. phlei*.

Fermentation studies were carried out in shake flasks on rotary shaker using soybean and glucose medium. The broth becomes deep orange yellow and maximum production (200 mgs. of crystalline antibiotic per litre of broth) takes place at 28°C. incubation after six days.



The antibiotic principle was isolated as red crystalline material with m.p. 254°C. (with decomposition). The antibiotic is distributed both in the broth and the mycelium and is readily soluble in acetone and chloroform, moderately soluble in benzene, ethyl alcohol, ethyl acetate and less soluble in ether, very sparingly soluble in carbon tetrachloride and water, and insoluble in petroleum ether. All the solutions including the broth show yellowish-green fluorescence in ultra violet light. On paper chromatography, the crystalline material appeared to be homogenous. Acetone and alcohol solutions gave a maximum absorption at 450 m $\mu$ . The compound appeared to be closely related to actinomycin group of antibiotics. Paper chromatography of acid hydrolysate revealed the presence of peptide chain attached to the chromatophoric group. Six distinct spots were obtained after ninhydrin development, of which three were provisionally identified as L-valine, L-proline and L-threonine.

In mice, 3.3 mg/Kg. was toxic both intravenously and intraperitoneally, but was tolerated when administered orally. A much less toxic, thermostable and completely water soluble degradation product was obtained, which while retaining most of the antibacterial activity was well tolerated in a single dose of 33 mg/Kg. when injected intravenously in mice. Further studies of both the toxic antibiotic and the less toxic water soluble degradation product are in progress.

The authors wish to express their grateful thanks to Dr. Ganapathi for the benefit of valuable suggestions and kind encouragement.

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## OCCURRENCE OF *MERULIUS HIMANTIOIDES* IN INDIA

B. K. BAKSHI, BALWANT SINGH AND T. CHOUDHURY

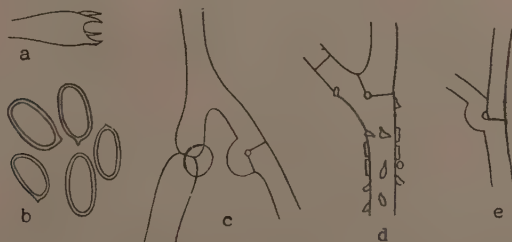
(Accepted for publication February 10, 1958)

Species of *Merulius* described under *M. himantioides* Fr., *M. silvester* Falck, *M. americanus* Burt, *M. brassicaefolius* Schw. and *M. terrestris* (Peck.) Burt are very close, so that their identity from sporophore and culture becomes difficult. Davidson and Lombard (1953), therefore, regarded them as synonyms of one species or as a species complex till further study can reveal them as distinct species. Allied to the above is *Merulius lacrymans* (Wulf.) Fr., which is mostly restricted to the indoors where it causes a severe dry-rot on timber. Interfertility tests between the monospore cultures of the above mentioned species may help to clear up the existing confusion in their identity. In this paper, the sporophore, culture and sexuality of *M. himantioides*, a new record from the Himalayas, are described.

*Merulius himantioides* Fr. This fungus was collected in the open forests in the Western Himalayas (Mundali, 8,000 ft., in the Chakrata division, Uttar Pradesh State) on the bark of fallen deodar (*Cedrus deodara*) and spruce (*Picea morinda*), and appears to be common in this locality.

*Sporophore.* Broadly effused, 2 - 15 cms. or more in diameter, smooth and white when young. Colouration (Ridgway, 1912) in mature areas consists of 'ochraceous tawny', 'tawny', 'verona brown', 'snuff brown' and 'bister'. Hymenial initials appear in form of reticulations which soon become convoluted into shallow folds, deepening on maturity, so that mature hymenium is reticulately porose but shallow (Pl. I, fig. 2), with pores angular, 0.5 - 2 mm. broad. Growing region remains white, the hyphae becoming fan-shaped and developing short strands (Pl. I, figs. 1 - 2), about 1 mm. broad, white at first, turning brown later. In section, 0.7 - 0.9 mm. thick, consisting of a light yellow hymenium of closely interwoven hyphae, a hyaline subhymenium of loosely interwoven hyphae,

Text-Fig. 1, a - e.



*M. himantioides* Fr. a. Basidia. b. Basidiospores. c. and d. Hypha, non-incrusted and incrusted respectively from sporophore. e. culture hypha. All x 1250.

and a deep yellow basal zone. Hyphae mostly hyaline, but yellow in areas attached to substratum, thin-walled, with (Text-fig. 1 d) or without (Text-fig. 1 c) incrustations, branched, with simple septa and abundant clamp connections, 3 – 7  $\mu$  broad. Basidia persistent (Text-fig. 1 a). Basidiospores yellow to rusty, usually guttulate particularly when fresh, double walled, ellipsoid with an apiculus (Text-fig. 1 b), 7.3 – 10 x 4.4 – 6  $\mu$ .

Dr. Harmsen, to whom the sporophore of the fungus was sent as *M. americanus*, states in a personal communication that it is better referred to under *M. himantioides*, since in the present collection, the subhymental layer is not gelatinized, while *M. americanus*, this is so.

**CULTURAL CHARACTERS.** Two isolates of the fungus have been obtained in culture. These isolates, Nos. 374 and 375, differ in growth rate and colour. 375 (Pl. fig. 3) grows faster and develops deeper shades of yellow on the mat than 374. Within an isolate again, colour is more pronounced at 18°C than at 26°C. The details of the fungus in culture are given below.

*Growth characters.* Radial growth on malt agar in 14 days in dark is 5 – 10 mm. at 18°C, 10 – 13 mm. at 23°C, and 12 – 15 mm. at 26°C. Mat cottony to cottony-silky, raised off the agar and extending to limits of growth. Advancing zone irregular. In 4 weeks, texture of mat unchanged, mycelium forming long, loose strands on agar and reach upper lid or roll over glass in tubes. Profuse water drops appear early (Pl. I, fig. 3,) which remain hyaline. Yellow shades ('pinard yellow', 'straw yellow', 'barium yellow') develop in 7 days over inoculum in the general white mat. In 4 weeks, same shades of colour spread over the mat which still contains white areas. In some transfers, yellow colour dominates when growth is checked. Undersurface bleached slightly. Odour strong. On gallic and tannic acid agars, diffusion zones absent, radial growth 9 – 15 mm. in former, trace on latter. On gentian violet agar, growth vigorous, medium not discoloured.

**HYPHAL CHARACTERS.** Hyphae hyaline, thin-walled, branched with abundant clamp connections (Text-fig. 1e), 2 – 8  $\mu$  broad.

Culture of *M. silvester* received from Dr. Gunther Becker, West Germany, and that of *M. himantioides* (Pl. I, fig. 4) isolated by Dr. Harmsen agree with each other and with the present isolates in all respects.

**SEXUALITY STUDY.** For this, single-spore cultures of the fungus were obtained in the following way:

Freshly discharged basidiospores were taken in sterile water, which was then poured over a plate containing malt-agar. After allowing the spores to settle, the water was drained out. Enough spores remained on the agar. The plates were incubated for 1 – 2 days, when the spores put forth short germ tubes. They were spotted under the low power objective



of the microscope and later cut out with the agar by a dummy objective, making sure that only one spore is included in the agar-disc thus cut out. This was transferred to tubes and monospore cultures were obtained.

		Ab							aB		AB		ab			
		1	3	4	6	7	12	14	2	13	5	9	10	8	11	15
Ab	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
aB	12	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	2	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
	13	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AB	5	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	9	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	10	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	8	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
ab	15	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
	11	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
	15	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-

Table 1. Pairing between monospore cultures of *M. himantioides*. + sign indicates presence of clamps. - sign their absence.

Single spore cultures of *M. himantioides* do not possess clamp connections in the hyphae. When two compatible cultures are mated, clamps develop on the mating mycelia. Clamps however do not form in incompatible pairings. Eight monospore mycelia of *M. himantioides* (nos. 1 - 8, table 1) were paired in all possible combinations on malt agar slants and examined for clamps when the two mating mycelia intermingled. The 8 monospores were found to fall into four sexual groups, AB, ab, Ab, and aB. Sexual groups of seven more monospore mycelia, nos. 9 - 15, were then determined by pairing with four monospore cultures (nos. 1, 2, 5 and 8) representing the four sexual groups. The results of mating experiments are given in table 1. The fungus is thus proved to be heterothallic and tetrapolar, which is also the case with *M. americanus* (Hwang, 1955).

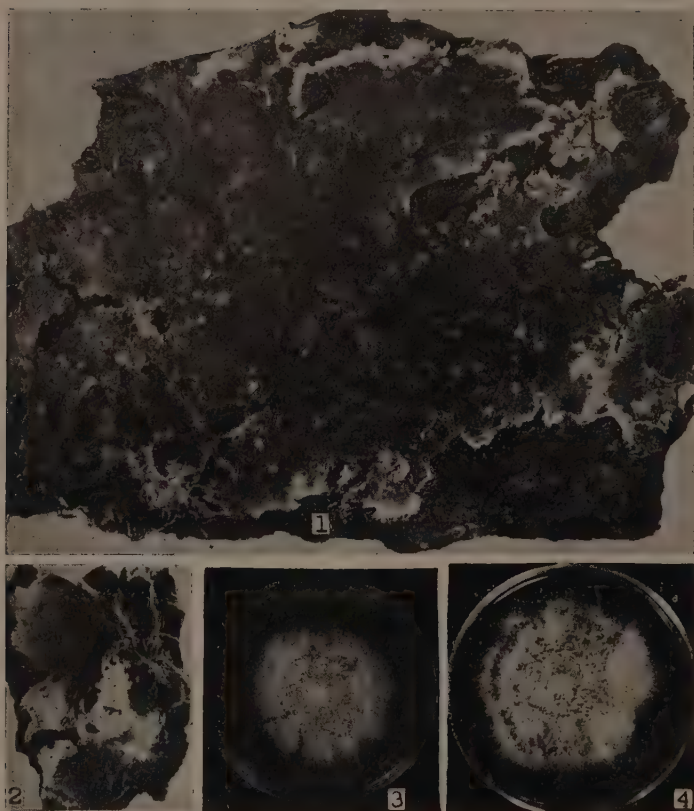
Our sincere thanks are due to Dr. Louis Harmsen, Denmark, for his valuable advice on the identity of the fungus.

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Plate 1.



*M. himantioides* Fr. Fig. 1. Sporophore on bark of deodar, general habit (x 1). Fig. 2. Same, showing porose hymenium and strands (x. 1.2). Fig. 3. 2-weeks old culture of the Indian isolate, no. 375 (x. 0.4). Fig. 4. 2-weeks old culture of the strain isolated by Dr. Harmsen.

# THE ROLE OF STALE PRODUCTS IN THE FORMATION OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY

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(Accepted for publication March 1, 1958)

**INTRODUCTION:** In two recent publications, Bedi (1956) has discussed the effect of some chemical factors on the growth and the sclerotial formation of the Punjab isolate of *Sclerotinia sclerotiorum* (Lib.) de Bary, causing the stem-rot disease of gram in the Punjab State. A mycelial mutant of this fungus, which had originated in a monophyphal culture of an isolate, originally obtained from wilted sunflowers by Dr. W. E. Sakston, Dominion Laboratory of Plant Pathology, Manitoba, Canada, failed to form sclerotia on different culture media, and different concentrations of nutrient glucose agar, as also under the influence of different sources of carbon and nitrogen in the culture medium. It produced only a few sclerotia on potato-dextrose agar at the restricted pH range of 4.1 to 5.9, and under the influence of uranium nitrate in the culture medium. At the above pH range, the very limited sclerotial formation had started only 15 to 17 days after the plates had been covered by the mycelium. On the other hand, in the case of the sclerotial Punjab isolate, the sclerotia had commenced forming only one day after the plates had been covered with the mycelial growth.

The role of stale products in inducing the mycelial mutant to form some sclerotia was, therefore, suspected.

Similarly, under the influence of uranium nitrate, the colonies of both the sclerotial Punjab isolate and the mycelial mutant had exhibited characteristics of staled growth.

The Canadian mutant is capable of producing abundant mycelial growth, which, ordinarily in the case of sclerotial races of *S. sclerotiorum*, is the basic building material for the formation of sclerotia. It evidently lacks the stimulus, which is necessary to convert its plentiful mycelium into sclerotia.

Also, in the case of the sclerotial Punjab isolate, when grown on media of moderate richness, only a peripheral ring of sclerotia is formed and the major central area of the colony, with a very thin mat of mycelium, remains devoid of them. At the periphery, where the mycelium accumulates, a relatively larger accumulation of stale products is expected.

**EXPERIMENTAL RESULTS AND DISCUSSION:** On the premise that stale products supply the necessary stimulus for the conversion of the mycelium into sclerotia, the following experiments were under-taken and the results obtained were spectacular.



1. EFFECT OF ADDING STALE PRODUCTS OF THE PUNJAB RACE TO ITS OWN CULTURES: For this experiment, the sclerotial Punjab race was grown in Petri plates containing potato-dextrose agar till it covered the surface of the medium, but had not as yet formed any sclerotia. The culture plates were divided into 4 lots of three each. One lot was kept without any treatment. From the remaining three lots of cultures, a disk about 2.25 cm. in diameter from the centre, and a ring about 0.75 cm. wide and about 1.5 cm. away from the edge, were removed. To one of these three lots, nothing was added. To the second and the third, 10 ml. of sterilized distilled water and 10 ml. of stale products of the Punjab race, grown on full-strength Richards' solution for 4 weeks, were added, respectively. The results of these treatments are shown in Table I and fig. 1.

A reference to Table I and also to fig. 1 shows very striking results. The addition of stale products (Fig. 1 - 4) has more than doubled the number of sclerotia, which are not only much larger and blacker, but are also more closely spaced than those produced in the remaining three treatments. The most significant point to be noted is the appearance of sclerotia in the central ring of mycelium, because ordinarily on media of moderate richness, like the one employed in this test, the central area remains free of sclerotia and only a peripheral ring of these bodies is formed.

TABLE I. Effect of adding stale products of the Punjab race to its own cultures before the formation of sclerotia

Treatment	Average number of sclerotia produced	Remarks
1. Control	30.0	Only one ring of sclerotia formed at the edge
2. One disk and one ring of medium with mycelium removed, but nothing added	20.0	-do-
3. One disk and one ring of medium with mycelium removed, and 10 ml. of distilled sterilized water added	28.3	-do-
4. One disk and one ring of medium with mycelium removed, and 10 ml. of stale products added	64.0	One ring of very closely-spaced sclerotia at the edge and numerous sclerotia irregularly scattered in the central ring of mycelium formed

Text-Fig. 1.



Fig. 1. Effect of adding stale products of the Punjab race to its own cultures before the formation of sclerotia.

1. Control.
2. One disk and one ring of medium with mycelium removed, but nothing added.
3. One disk and one ring of medium removed and 10 ml. of sterilized distilled water added.
4. One disk and one ring of medium with mycelium removed and 10 ml. of stale products of the Punjab race added. In this case, a remarkable increase in the number of sclerotia may be noticed. Also, notice that sclerotia in the peripheral ring are more closely spaced, are blacker and larger than those in the peripheral rings under other treatments and the control. The most significant thing, however, is the appearance of numerous sclerotia in the central ring, which under other treatments is totally devoid of them.

The writer offers the following explanation for the absence of sclerotia in the central portion of the culture on media of moderate nutritive status, like potato-dextrose agar. The colony grows very fast during the first 2 or 3 days and exhibits little or no staling, and by the end of this period the plate is generally covered. During the rapid radial advance of the

colony, the mycelial mat produced is extremely thin, and for want of adequate amount of mycelium, it is unable to support the growth of sclerotia. At the edge, however, where the advance of the colony is checked, the mycelium has no alternative but to pile up and is thus abundant enough to supply the material needed for the formation of sclerotia. In the central area with thin mycelial mat the amount of stale products is expected to be very small, and at the periphery, where the mycelium accumulates, a relatively larger accumulation of stale products is expected, and these stale products seem to supply the necessary stimulus for the conversion of the mycelium into sclerotia. It was very interesting to note that in this experiment, when stale products had been added, the first effect was the abundant development of fluffy mycelium, which very soon rounded up into white, raised-up, button-like structures eventually turning black and forming the definite hard resting bodies, the sclerotia. Some of these mycelial button-like outgrowths may be seen in the central ring of fig. 1 - 4 even after the formation of sclerotia.

This experiment, in addition to showing the great stimulatory effect of stale products on sclerotial formation, lends further support to the hypothesis that sclerotia can occur only in those regions of cultures, where the mycelium crowds densely or piles up due to one cause or the other.

2. EFFECT OF ADDING STALE PRODUCTS OF THE PUNJAB RACE TO CULTURES OF THE CANADIAN MYCELIAL MUTANT RACE: As in the last experiment, the mycelial mutant race was grown on potato-dextrose agar till it covered the plates. The plates were divided into 4 lots of 3 each. One lot was kept without any treatment to serve as control. From the remaining three lots, one central disk and one ring of agar with mycelium were removed, as in the foregoing experiment. To one of these three lots, nothing was added. To the second, 10 ml. of stale products of the mycelial mutant race itself, and to the third a similar amount of the stale products of the sclerotial Punjab Race were added. Both the races had been allowed to grow on Richards' solution of full strength for 4 weeks for obtaining the stale products used in this test.

The striking results obtained as the result of these treatments may be seen in fig. 2.

It may be noticed that in the control plate, i.e. in the plate where nothing is added, as also in that where its own stale products have been added, the mycelial mutant race remains totally devoid of sclerotia as usual. But where the stale products of the sclerotial race from Punjab are added, the miracle happens, and a very large number of small sclerotia lining the outer edge of the central mycelial ring and the inner edge of the peripheral ring appear. This test also proves that it is only the stimulatory effect of the stale products of the sclerotial Punjab race that is instrumental in the formation of sclerotia by the mycelial mutant race, and not necessarily the nutrients contained in the stale products, because the nutrients in its own stale products have failed to do the job, and furthermore, this race, even when grown on extremely concentrated and rich media, like 4 times the full strength of nutrient glucose medium or milk



Text Fig. 2



Fig. 2. Effect of addition to cultures of the mycelial mutant race (C.M.) its own stale products and those of the sclerotial race from Punjab (P.). Notice the formation of sclerotia by this race, where the stale products of the Punjab race (P) have been added and their absence where its own products have been used. The stales used were four weeks old.

agar and homogenized egg, does not produce sclerotia. As to what particular substance there exists in the stale products of the sclerotial Punjab race, which stimulate this ordinarily mycelial race to form sclerotia has not been determined as yet. This, however, is a fertile field for research. This experiment was repeated, and the stale products of the sclerotial race from Punjab, this time grown on Richard's solution for 8 weeks instead of 4, were used, presuming that the older stale would be stronger and capable of greater stimulation. The expected happened, as may be seen in fig. 3. This time, the sclerotia produced are much larger and more well-defined than where a four-week-old stale had been employed in the previous experiment. It was also interesting to note that the stale products of the parent sclerotial race on sunflower from Canada also behaved similarly to those of the Punjab race in stimulating sclerotial formation by the mycelial mutant race.

Text Fig. 3

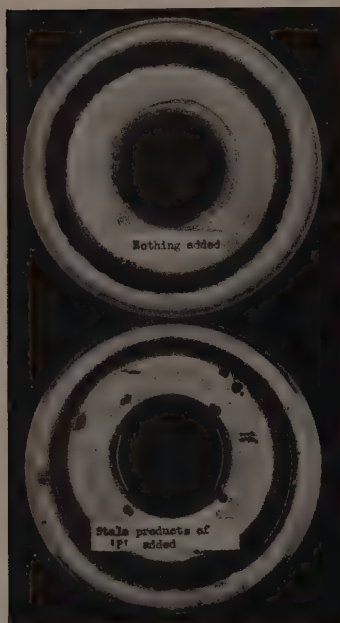


Fig. 3. Effect of adding 8-week-old stale products of the Punjab race (P.) to the culture of mycelial mutant race. Notice the formation of larger and more well-defined sclerotia as the result of adding stale products of twice the age. Compare with fig. 2, where 4-week-old stale products have been used.

Asthana and Hawker (1936) stimulated perithecial formation in *Melanospora destruens* by adding stale products of certain fungi to the culture medium. The stimulatory effect of stale products was considered by them as possibly due to reduction in food concentration of the staled medium, presence of inhibiting substances or presence of certain vitamins. Brown (1925) noted in certain strains of *Fusarium* a correlation between intense staling and intense sporulation.

3. EFFECT OF REMOVING STALE PRODUCTS FROM CULTURES: For this purpose also, the Punjab race was grown on potato-dextrose agar till it covered the plates. The plates in this test were divided in to 5 lots of 3 each. One lot was kept without any treatment to serve as control. From the remaining 4 lots, one peripheral ring of agar with mycelium about 1 cm. wide was removed from the edge of the colonies. From two out of these 4 lots without the peripheral ring, an additional disk was removed from the centre. Sterile Richards' solution of quarter strength was added and changed every 4 hours for 3 days, excepting the 8-hour period from 12 P.M. to 8 A.M., to leach out the stale products from the

cultures in two lots, while the control and other two lots were not so treated. Richard's solution instead of water was used in an attempt to counteract the effect of nutrients that may be leached out from the cultures along with the stale products. The results are striking in this test also, as shown in fig. 4.

Text Fig. 4



Fig. 4. Prevention of sclerotial formation in cultures of the Punjab race by the removal of stale products from them.

In the control plate a ring of black sclerotia on the edge of the colony against the glass is clearly seen. In the case of the two plates from which a ring, and a ring and a disk have been removed, but the stale products have not been leached out, the sclerotia can be seen clearly in the white piled-up peripheral mycelium. On the other hand, in the plate from which only the peripheral ring has been removed and the cultures had been treated with fresh Richards' solution in an attempt to leach out the stale products, sclerotia are no doubt absent, but still there is some attempt on the part of the culture to form button-like agglomerations of mycelium on the edge, perhaps due to the incomplete leaching-out of the stale products. But the formation of sclerotia has been completely inhibited in the plate from which the stale products have been more completely removed by flooding both the periphery and the centre with Richards' solution.

#### SUMMARY

As a result of the addition of stale products of the Sclerotial Punjab race to its own cultures, there occurs a tremendous increase

in the number of sclerotia. Even their size increases and their formation is hastened. Even the central portion of the culture, which ordinarily remains devoid of sclerotia, produces them abundantly. The first effect of the addition of stale products is the increased development of fluffy mycelium, which very soon rounds up into white, raised-up button-like structures turning black to form sclerotia.

The mycelial Canadian mutant race, which ordinarily does not form sclerotia, is also stimulated to do so, when the stale products of the sclerotial Punjab race are added to its cultures. Its own stale products, however, do not serve this purpose.

Sclerotial formation in the case of the Punjab race, which normally produces sclerotia, is prevented if the stale products from its colonies are removed, as soon as formed.

ACKNOWLEDGEMENTS. The work was carried out in the Division of Plant Pathology, College of Agriculture, University Farm, St. Paul, Minnesota (U.S.A.), and forms a small part of the thesis submitted for the Degree of Doctor of Philosophy. The writer is extremely indebted to Dr. E. C. Stakman, Head of the Division of Plant Pathology, and Botany, under whose inspiring guidance the work was carried out. Grateful thanks are also due to the Punjab Government for the award of a scholarship enabling the writer to pursue his studies in the United States of America.

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# EFFECT OF ULTRA-VIOLET RADIATION OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY ON THE SPEED OF THEIR GERMINATION AND APOTHECIAL DEVELOPMENT

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Ramsey and Bailey (1930) observed a definite stimulation of spore production in cultures of *Macrosporium tomato* and *Fusarium cepae* on exposure to ultra-violet radiation. Similarly, Bailey (1932-33) observed a great hastening of sporulation in *Fusarium culmorum*, as a sequel to ultra-violet radiation. Acceleration in the germination of seeds of certain crop plants due to ultra-violet rays has been recorded by Singh, Kapoor and Chaudhari (1936). The writer also in his studies of *Sclerotinia sclerotiorum* determined the effect of ultra-violet radiation of sclerotia on the speed of their germination and apothecial development. The results obtained were very spectacular, and are described in this paper.

For these studies, sclerotia were produced on potato-dextrose agar in Petri plates at a temperature range of 20° - 22°C. When they were three weeks' old and had matured fully, they were exposed after removing the covers of the plates to ultra-violet radiation from a 4-watt germicidal lamp at a distance of 10 inches for 30 minutes, 1 and 2 hours, respectively.

There were triplicate plates with sclerotia for each period of exposure. Sclerotia in a similar number of plates were not irradiated and served as controls. Immediately, the treated sclerotia, along with the untreated ones, were floated on water (Bedi, 1956) contained in Erlenmeyer flasks, which were placed under conditions suitable for the development of apothecia, i.e., a temperature range of 15° - 20°C., and a daily photo-period of 12 hours. The experiment was continued for a period of six weeks and the results obtained are recorded in Table I.

TABLE I. Effect of ultra-violet radiation of sclerotia on the speed of their germination and apothecial development, as recorded at the end of six weeks.

Period of irradiation	Days after which the sclerotia began to germinate	Percentage* of sclerotia with stipes only	Percentage of sclerotia with normally expanded apothecia	Percentage total germination	Average number of apothecial processes per sclerotium
		(A)	(B)	(A)+(B)	
0 (Control)	32	60.8	17.4	78.2	1.1
30 minutes	25	59.1	32.4	91.5	2.3
1 hour	12	0	100	100	2.7
2 hours	16	30.2	69.8	100	2.0

\*Average of 3 replicates.

Data set out in Table I show that the exposure of sclerotia to ultra-violet radiation hastens their germination. An exposure of one hour seems to be the best. In this case, the sclerotia begin to germinate by putting forth stipes in as short a period as 12 days, against 32 days in the un-irradiated (control) series. This reduction in the period required for their germination, after they are floated on water, amounts to 62.5 per cent and is spectacular. An exposure of 2 hours, though inferior to that for one hour, cuts down the period required for germination to 50 per cent. Even a 30 minutes' exposure reduces this period by about 22 per cent.

Total germination of sclerotia at the end of six weeks is 100 per cent under the influence of both one and two hours' exposure as compared to 78.2 per cent in the un-irradiated control series; but there is another very important difference. This is the formation of 100 per cent normally expanded apothecia under one hours' exposure against 69.8 per cent in the case of 2 hours' exposure and only 17.4 per cent in the control series. Though the mere putting-forth of stipes by the sclerotia implies germination, yet it is not effective germination, which must result in the formation of apothecia and their normal expansion. It is the normally-expanded apothecia, which expose the hymenium bearing the asci for ascospore dissemination by air currents. Though in the case of control series the total germination is 78.2 per cent, the effective germination, resulting in the formation of normally-expanded apothecia, is as low as 17.4 per cent.

It may be further noticed that the ultra-violet radiation not only hastens the germination of sclerotia, and increases the development of normal apothecia, but also greatly increases the emergence from a sclerotium of apothecial processes (stipes), at the end of which apothecia are borne. Such an increase is advantageous to the pathogen in its struggle for existence, because of the correspondingly increased ascospore production. It may be observed that even an exposure of 30 minutes in this experiment has more than doubled the average number of stipes emerging from a sclerotium. One hour's exposure, which has induced 100 per cent effective germination of sclerotia, has also resulted in the maximum increase (145.4 per cent) in the number of apothecial processes emerging from a single sclerotium.

The periods of exposure of sclerotia to ultra-violet radiation are rather long, and they were considered to be necessary in view of the jet black colour, and very hard and horny rind, which seem to make the sclerotia very resistant to external influences. It may be appreciated that even an exposure of as long a period as two hours, instead of injuring the sclerotia, hastens their germination very much—only 16 days being required to initiate germination instead of 32 days in the case of control series. It further increases the formation of apothecia from 17.4 to 69.8 per cent and almost doubles the number of the apothecial processes emerging from a sclerotium. Stevens (1931) also states that fungi having dark spore walls are more resistant to ultra-violet radiation than those with light spore walls.

## SUMMARY

Exposure of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary to ultra-violet radiation for periods ranging from 30 minutes to 2 hours is not harmful. On the other hand, it very much hastens their germination, and increases the number of normally-expanded apothecia to a great extent. The number of apothecial processes emerging from a single sclerotium also increases greatly as a sequel to the treatment.

One hour of exposure constitutes the optimum period for inducing the germination of sclerotia in the shortest period of time (12 days instead of 32 days) after they are floated on water. This period of exposure also results in 100 per cent effective germination of sclerotia, i.e., their stipes bear normally-expanded apothecia, against only 17.4 per cent of them in the un-irradiated series. The number of stipes emerging from a single sclerotium also increases by about 145 per cent as the result of this period of exposure.

ACKNOWLEDGEMENTS. The work was carried out in the Division of Plant Pathology, College of Agriculture, University Farm, St. Paul, Minnesota (U.S.A), and forms a small part of the thesis submitted for the Degree of Doctor of Philosophy. The writer is extremely indebted to Dr. E. C. Stakman, Head of the Division of Plant Pathology, and Botany, under whose inspiring guidance the work was carried out.

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EFFECT OF OTHER MICRO-ORGANISMS ON THE GROWTH  
AND SCLEROTIAL FORMATION OF *SCLEROTINIA*  
*SCLEROTIORUM* (LIB.) DE BARY

KISHAN SINGH BEDI

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At an early stage of investigations on *Sclerotinia sclerotiorum*, it was noticed by the author that its cultures were highly susceptible to unfavourable influences of a number of other organisms. The sclerotia of this fungus, obtained from Punjab, were found to have been attacked to the extent of 94 per cent by a *Fusarium* sp. A culture of *Trichoderma lignorum* (Pers. Ex. Fries) Bisby, obtained from Dr. C. M. Christensen, Division of Plant Pathology, St. Paul, Minnesota, was observed in preliminary studies to over-run the colonies of this pathogen, if grown in the same receptacle with it at room temperature (22° to 25°C.). Similarly, an isolate of *Bacillus subtilis* group isolated from soil by Anwar (1949) was deleterious to its growth and sclerotial formation at room temperature. To appreciate the extent of antibiotic action, which this bacillus is capable of exerting towards this pathogen, fig. 1 may be examined. The upper Petri plate shows culture of the sclerotial Punjab race of this fungus growing in the absence of the bacillus. The entire culture is covered with a white mycelial felt having a ring of black sclerotia on the periphery. The lower culture plate shows the same race surrounded by four colonies of the bacillus. Under its influence, the culture of the race has undergone such a profound change in its morphology that it bears no resemblance at all to the normal white colony on the left. There is practically no aerial mycelium and the thin appressed fungal mat is almost black on the periphery. This extreme pigmentation of the otherwise-white culture is probably due to a toxin produced by the bacillus. Production of intense pigmentation in the cultures of *Aspergillus niger*, growing in contact with *Penicillium africanum*, has also been recorded by Doebelt (1909). According to him, the pigment accumulates in the mycelium of *A. niger*, which may thereby be killed. Death of cultures of the Punjab race in about 3 weeks at room temperature (22° to 25°C.) under the action of *B. subtilis* has also been observed by the author. That *B. subtilis* actually produces anti-fungal substances, has been shown by Michner and Snell (1949). It may be noticed that, in addition to the suppression of the aerial mycelium and the intense pigmentation of the colony, sclerotial formation has also been totally inhibited by the bacillus. The inhibiting substance is thermo-labile, because it is not effective after autoclaving (Fig. 2). In the upper culture plate, two filter paper disks soaked in a filtrate of the cultures of *B. subtilis*, sterilized by passing through a Seitz filter, were placed on two opposite points. The total suppression of sclerotia up to a considerable distance on both sides of each filter paper disk may be noticed. In the lower culture plate, similar filter paper disks, soaked in autoclaved filtrate, were placed. The mycelium of the fungus, in place of being inhibited, has over-run the disks and has formed



two definite rings of sclerotia around them. This shows that the toxic principle of the filtrate has been destroyed by heat sterilization, and the sclerotial formation has been stimulated in the regions, where the filter paper disks have been placed. This phenomenon will be investigated further.

Text Fig. 1



Text Fig. 2



Fig. 1. In the upper plate, is a normal, white colony of the Punjab race of *Sclerotinia sclerotiorum* with a ring of black sclerotia near the periphery. In the lower plate is a culture of the same race surrounded by four colonies of *Bacillus subtilis* growing at room temperature ( $22^{\circ}$  to  $25^{\circ}$  C.). Note the dark pigmentation of the colony, its atrophied size, the almost total suppression of aerial mycelium and complete inhibition of sclerotial formation, as a sequel to the strong antibiotic action of the bacillus.

Fig. 2. In the upper culture plate, two filter paper disks, soaked in a filtrate of the cultures of *B. subtilis*, sterilized by passing through a Seitz filter, were placed on two opposite points. The total suppression of sclerotia upto a considerable distance on both sides of each filter paper disk may be noticed. In the lower culture plate, similar filter paper disks soaked in boiled (actually autoclaved) filtrate were placed. Note not only the total loss of the toxic action of the filtrate due to heat sterilization, but also its stimulating action on sclerotial formation, as evidenced by the formation of two definite rings of sclerotia surrounding the filter paper disks, which also have been over-run by the mycelium of the fungus.

The antibiotic effect of *B. subtilis* towards other fungi has been recorded by other workers also. Its adverse effect on *Helminthosporium sativum* has been pointed out by Anwar (1949), and on *Fusarium udum* by Vasudeva (1949).

That *Sclerotinia sclerotiorum* is adversely affected by a number of organisms is evident, and it has been reported to be parasitized by a *Coniothyrium* species by Campbell (1947), but it has a wide geographic distribution, and is the cause of very destructive diseases of scores of economic plants. It must, therefore, possess means that enable it to compete successfully with other organisms in nature. The dominance of some organisms over it under one set of conditions does not give a correct picture. It is well-known that both the chemical and physical factors of the environment affect the various organisms occurring together and competing with one another. Under a particular environment, the organism, that finds conditions more suitable for its development, will grow more rapidly and form its fruiting bodies or other organs, that are necessary to its survival, more quickly than the other, which may be suppressed. It has not as yet been possible to study the effect of several factors of the environment on *S. sclerotiorum*, when grown in mixed cultures with other organisms. Only the temperature factor has been studied and its effect is described in this paper,

The sclerotial Punjab race was grown on potato-dextrose agar in plates singly, and in association with *Fusarium* species from Punjab, *Trichoderma lignorum* and *Bacillus subtilis* at temperatures ranging from 5° to 30°C. One inoculum of the Punjab race in a plate was surrounded by four inocula of any one of the other organisms.

Both the growth and the sclerotial formation of the Punjab race are profoundly affected in association with other organisms at different temperatures and the pertinent data, as recorded at the end of 15 days, are set out in Table I, and are illustrated in Fig. 3.

TABLE I. Effect of other organisms on the formation of sclerotia by the Punjab race at different temperatures, as recorded at the end of 15 days.

Treatment	Temperature in degrees Centigrade					
	5	10	15	20	25	30
	Number of sclerotia formed*					
1. Punjab race grown singly	0	30.3	54.0	29.3	35.6	20.0
2. Punjab race grown with <i>Bacillus subtilis</i>	0	19.6	17.0	7.0	0	0
3. Punjab race grown with <i>Trichoderma lignorum</i>	0	27.3	13.0	3.3	0	0
4. Punjab race grown with <i>Fusarium</i> sp. from Punjab	0	6.3	12.3	7.0	7.6	0

\*Average of 4 replicates

Text Fig. 3



Fig. 3. The effect of *Bacillus subtilis*, *Trichoderma lignorum*, and *Fusarium* which were isolated from sclerotia of *Sclerotinia sclerotiorum* from Punjab, India, on the growth and sclerotial formation of the Punjab race at temperatures indicated ( $^{\circ}\text{C}$ ).

It may be noticed that when grown singly, the Punjab race has covered the surface of the medium at all temperatures except 30°C., at which it produces, as usual, a small staled colony with a wavy margin. Sclerotia are formed at all temperatures except 5°C., at which it takes about 4 weeks to form them. As the experiment had been terminated at the end of 15 days, they are not visible in fig. 3.

When grown with *B. subtilis*, its growth and sclerotial formation vary within very wide limits.

At 5°C., the bacillus does not grow at all. The race is growing fairly well, though its linear spread and mycelial development seem to be appreciably affected, probably due to some toxins having diffused into the medium from the inocula of the bacillus. In a previous test, which was extended over a longer period, the fungus had actually over-run the inocula of the bacillus.

At 10°C., though the bacillus has made only a slight growth, yet it has produced four definite small zones of inhibition. Though the mycelial growth of the race has not been appreciably diminished, yet the sclerotial formation has been reduced from 30.3 to 19.6 (Table I), and this is a considerable reduction.

At 15°C., the bacillus has grown still further, but its colonies are still small. The zones of inhibition, however, are now very clear-cut and several times larger than those at 10°C. Though the mycelial development of the colony has also diminished visibly, yet the decrease in the number of sclerotia is far too large, there being only 17 of them on the average against 54 in the absence of the bacillus.

At 20°C., the inhibition zones have widened very much. Though there is an appreciable decrease in the amount of aerial mycelium, yet the reduction in sclerotial formation is too much, the average number of sclerotia having diminished from 29 in the control colonies to only 7 in those under the influence of the bacillus. At 25°C., the bacillus has got the upper hand, as it has not only suppressed the development of the aerial mycelium and the sclerotia totally, but has also changed the morphology of the colony beyond recognition by the production of intense pigmentation. The colony, as may be noticed, has turned dark and is almost black at the edges.

At 30°C., the bacillus has gained full dominance covering the entire plate in total disregard of the inoculum of the Punjab race.

In brief, it may be stated that the Punjab race, though adversely affected to a greater or lesser degree at temperatures varying from 5° to 20°C., can never-the-less, maintain itself in competition with *B. subtilis* at this temperature range. At the higher temperatures of 25° to 30°C., it, however, loses its battle altogether. It was observed that the bacillus had actually killed it in about 3 weeks at 22° to 25°C., despite the fact



that this temperature range is very suitable for the growth of the fungus. It was also seen by an actual inoculation experiment with sun-flower and gram plants that the Punjab race altogether failed to cause infection in the presence of this bacillus.

When grown with *Trichoderma lignorum*, which has been shown to be highly destructive to *Sclerotinia sclerotiorum* by Hino (1935) and to various other phytopathogens by Hino and Endo (1940), the Punjab race behaves very much in the same way as in competition with *B. subtilis*, with the difference that at 10°C., it suppresses *T. lignorum* totally and produces its mycelial growth and sclerotia normally. *T. lignorum* begins to exercise its adverse influence at temperatures of 15°C., and above, so much so, that at 20° it considerably cuts down the mycelial growth of the race and reduces its sclerotial formation to an extremely low figure of only 3.3 against 29.3 obtained in its absence (Table I).

At 25° and 30°C., *T. lignorum* overgrows the race, as if the latter is not at all present.

When grown in association with *Fusarium* sp., the Punjab race tells a different story. It finds the *Fusarium* as a much more formidable competitor than either of the other two organisms mentioned above. They, at least, are suppressed or rendered ineffectual by it at 10°C., or below.

The *Fusarium*, on the other hand, follows it closely over a very wide range of temperatures, viz., 5° to 25°C., and at 30°C., which temperature happens to be favourable to its own growth, but unfavourable to that of *S. sclerotiorum*, it naturally cannot but dominate. At temperatures other than 30°C., the two fungi seem to grow almost equally well and appear to be mutually compatible and do not encroach upon each other. The considerable reduction in the number of sclerotia of the Punjab race at various temperatures other than 30°C. seems to be due to spatial reasons and the consumption of nutrients by the colonies of *Fusarium* in their normal course of growth. It is interesting to note that the reduction in the amount of sclerotial formation at temperatures ranging from 10° to 25°C., is almost the same. This shows that, unlike the other two organisms, there is no progressively accentuated adverse effect produced by it with the rise in temperature.

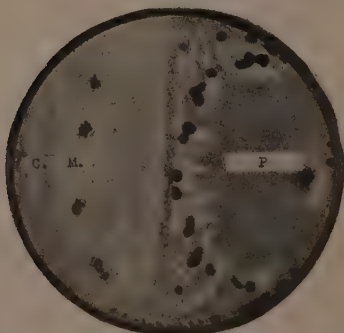
**EFFECT OF GROWING THE PUNJAB RACE AND THE CANADIAN MUTANT RACE TOGETHER.** The mycelial mutant race of *S. sclerotiorum* forms sclerotia only under special conditions, one of which is the addition of stale products of the sclerotial races to its cultures. As a corollary to this result, it was considered logical to ascertain the influence of growing the mycelial race in association with the sclerotial Punjab race. For this purpose, the inocula of the two races were placed in the same plate of potato-dextrose agar at two opposite points near the edge. Their cultures advanced towards each other and met in the middle of the plate, as shown in fig. 4. It should be carefully noted that in this case there is no piling-up of mycelium as always occurs, when two or more colonies of the Punjab race meet (Bedi, 1956). It appears that the mycelia of the two races do

not intermingle, though they come close to each other. A definite line of demarcation separating the two races is clearly discernible and the Punjab race forms sclerotia not at this line of demarcation, but within the region occupied exclusively by its own mycelium.

Within a few days after the colonies of the two races had met, the mycelial race also formed a few sclerotia, which may be seen in fig. 4. There is every reason to believe that this is due to the diffusion of some of the sclerotial Punjab race's metabolic products containing some stimulatory substances. The exact nature of these substances has not been determined as yet. May be, the mycelial race is deficient in certain vitamins like biotin, necessary for sclerotial formation and those vitamins, for which the Punjab race may be autotrophic, are supplied by it to the former, when grown together with it. This assumption receives support from the work of Barnett and Lilly (1947), who state that *Sclerotinia sclerotiorum* is at least autotrophic for biotin. The sclerotial race of *S. sclerotiorum* from Canada, which had given rise to the mycelial mutant race under study, was also effective in inducing the latter to form sclerotia, when grown in association with it.

It has been noticed by several workers that the formation of fruiting bodies by certain fungi or strains of fungi, which under ordinary conditions remain sterile, takes place when they are grown in association with other organisms. Miss McCormick (1925) has reported that though *Thielavia basicola* sometimes forms perithecia, when cultured alone, it is greatly stimulated in perithecial production by the presence of *Thielaviopsis basicola*, *Cladosporium fulvum*, *Aspergillus umbrosus*, *Eurotium amstelodami* and *Fusicladium pirinum*. According to Barnett and Lilly (1947), *Sordaria fimicola*, which does not produce biotin, but requires it for perithecial formation produced perithecia on a vitamin-free medium when grown jointly with fungi, that are autotrophic for biotin, such as *Phycomyces blakesleanus*,

Text Fig. 4



Text Fig. 5

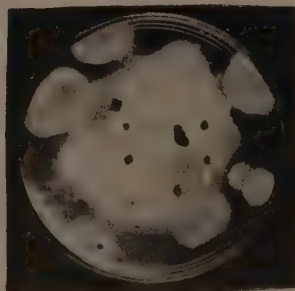


Fig. 4. Sclerotial formation by the non sclerotial mutant (C.M.) induced by growing it in association with the Punjab race (P.), which normally produces sclerotia.  
Fig. 5. Sclerotial formation by the mycelial mutant race of *S. sclerotiorum*, as the result of accidental contamination with saprophytic fungi.

and *Monascus purpureus*. The effect was similar to that when pure biotin was used.

Similarly, Wilson (1927) states that in the presence of certain fungi, *Venturia inaequalis* forms perithecia more abundantly than otherwise. Likewise, Gupta (1933) reports that two infertile saltants of *Cytosporina ludibunda* produced pycnidia, when the two were grown together.

The production of some sclerotia by the mycelial mutant race in a Petri plate of potato-dextrose agar, as a result of accidental contamination with *Penicillium* and *Aspergillus* species, is shown in fig. 5. Here also, the production of sclerotia is most probably due to the diffusion of certain stimulatory metabolic products of the contaminants.

#### SUMMARY

The growth and the sclerotial formation of the Punjab race of *Sclerotinia sclerotiorum* (Lib.) de Bary are greatly influenced by other organisms. *Bacillus subtilis* exercises a strong antibiotic effect on the colonies of this race at room temperature (22°–25°C.), totally inhibits sclerotial formation and eventually kills the fungus. At the lower temperature range of 10° to 20°C., although its sclerotial formation suffers considerably, the fungus can maintain itself in competition with the bacillus, and at 5°C. actually over-runs the latter.

In competition with *Trichoderma lignorum* at 10°C., the *Sclerotinia* fungus suppresses the former and produces its normal quota of mycelial growth and sclerotia. *T. lignorum* begins to exercise its adverse effect on the *Sclerotinia* fungus at the temperature of 15°C., and above. At 20°C., it suppresses sclerotial formation by the latter to a great extent and at 25° and 30°C., totally suppresses it.

The *Fusarium* species, which was isolated from the sclerotia obtained from Punjab, though not antagonistic to *S. sclerotiorum*, is a tough competitor of the latter because of its very wide latitude of temperature requirements. It closely follows the *Sclerotinia* over the wide temperature range of 5° to 25°C., at which it reduces the sclerotial formation of the latter in proportion to the surface of the culture medium occupied by its colonies. It is only at 30°C., which temperature is not favourable to the growth of the *Sclerotinia* fungus, that the *Fusarium* suppresses its sclerotial formation altogether.

The mycelial Canadian mutant of *Sclerotinia sclerotiorum*, despite the abundance of mycelium it produces, remains totally devoid of sclerotia on most of the culture media and under different environmental conditions. It, however, produces some well-developed sclerotia, when it is grown in association with the sclerotial Punjab race or its own sclerotial parent.

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## WATERMELON WILT IN BOMBAY STATE CAUSED BY *FUSARIUM OXYSPORUM* f. *NIVEUM* (E.F.S.) S. & H.

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**INTRODUCTION.** Watermelon (*Citrullus vulgaris* L.) is an important crop in Bombay State and is usually grown in the *rabi* season on the banks of rivers or in rice fields after harvest of rice. The total area under this crop in Bombay State is about 26,000 acres including the area under muskmelon (*Cucurbita moschata* Duchesne). A destructive wilt disease of watermelon was reported from Dorli in Kalyan Taluka of Thana district in January, 1955; the damage ranged from 20 to 30 percent. A species of *Fusarium* was isolated from wilted plants and since a *Fusarium* wilt of watermelon had not been recorded in India so far, it was thought worthwhile to study the causal organism with a view to ascertaining its identity.

**SYMPTOMS.** In the field, affected vines suddenly wilt and do not recover. Wilting starts in one or more branches and in a few days the whole vine is involved. The disease may appear at any stage of growth of the vine but is most severe at fruit setting. If younger plants are infected, they remain stunted with short internodes; such vines never bear fruits but die prematurely. The vascular system of wilted vines shows the characteristic browning of vessels as in other *Fusarium* wilts.

**PATHOGENICITY OF THE FUNGUS.** *Isolation.* Numerous isolations were made from roots of wilted watermelon plants by the usual method of planting on potato-dextrose-agar. The plates were incubated at 30°C. Fungus growth was noticed after 3 days. Transfers from these plates were made on to P.D.A. slants.

*Inoculation.* For inoculation experiments, the fungus was grown on Richards' medium for about 10 days and the fungus growth was uniformly mixed with sterilised soil which was then stored in a cool place with constant stirring and watering for about one month to allow maximum growth of fungus. This soil was then filled in earthen pots (4 inch size) which were previously sterilised by dipping in 5 per cent copper sulphate solution and washed in water; 5 seeds of watermelon (local variety) were sown in each pot and an equal number of pots containing sterilised soil was sown as control. The pots were kept on a glasshouse bench where the mean air temperature fluctuated between 25° - 30°C.; 22 days after sowing of seeds, the seedlings started wilting in the pots containing inoculated soil; 100 percent wilting was noticed after 1½ months after planting while plants in control pots remained healthy. Stunting of plants in the case of affected plants was also common.

*Reisolation.* Reisolation made from wilted plants from the infection experiment, yielded the fungus which was indistinguishable in all respects from the original culture used for inoculation.

*Morphology.* Mycelium is septate, colourless to fleshy, pink or purple. On potato-dextrose-agar sclerotial bodies are produced, which measure upto 3-6 mm. in diameter. But sclerotia disappear or become colourless when cultures become old. Microconidia 0-2 septate, straight or curved, are produced freely in the aerial mycelium. Macroconidia are usually 3-5 septate, elongated, almost cylindrical to fusiform; falcate tapering at both ends. Sporodochia are formed on affected plants in nature and pinnates are produced on a few media. The apices of macroconidia are somewhat constricted, abruptly bent or conical, bases truncated, conical or pedicellate. Chlamydospores, both terminal and intercalary, are produced on many media and are spherical or oval, one celled or two celled.

**IDENTITY OF THE FUNGUS.** The presence of terminal and intercalary chlamydospores, delicate walls and septation of macroconidia, and abundance of single, one-celled, ovoid microconidia, place the watermelon wilt fungus in the section *Elegans* of the genus *Fusarium*.

The measurement of the micro and macroconidia of the fungus under study on potato-dextrose-agar and those of *Fusarium bulbigenum* var. *niveum* as given by Wollenweber and Reinking (1935) and by Doidge (1939) are given in Table I.

TABLE I. Comparative spore measurements of the water melon wilt *Fusarium* under study and *Fusarium bulbigenum* var. *niveum* given by Wollenweber and Reinking (1935) and by Doidge (1939).

Sr. No.	Item	No. of septa	<i>Fusarium</i> under study (microns)	Wollenweber & Reinking (Microns)	Doidge (microns)
1. Microconidia		0	9.04 x 3.03 (7.05-12.56 x 2.47-3.52)	8.6 x 2.8 (6.7-11.0 x 2.2-3.3)	Mostly 6.7-11.0 x 2.2-3.3 (5-12 x 2.0-4.5)
		1	15.95 x 3.16 (13.72-20.04 x 2.83-3.15)	16 x 2.9 (12-18 x 2.7-3.0)	Mostly 12-18 x 2.7-3.0 (10-24 x 2.5-5.0)
		3	33.89 x 3.83 (30.1-45.24 x 2.9-4.23)	34 x 3 (29.40 x 3.1-4.0)	Mostly 29-40 x 3.1-4 (24-50 x 2.7-7.0)
2. Macroconidia		5	46.3 x 3.92 (42.8-58.25) x 3.56-4.8	47 x 3.6	Mostly 43-56 x 3.4-4.3 (40-66 x 3.5)
		1	8.2 (5.8-11.0)	5.10	5.10
3. Chlamydospores		2 celled	14.3 x 8.4 (12-16 x 7-9)	12.6 x 7	12-15 x 7

From the measurement of conidia, type of chlamydospores and characteristic growth on oatmeal agar and steamed rice the fungus is indistinguishable from the fungus described by Wollenweber and Reiking (1935), and by Doidge (1939) as *Fusarium bulbiginum* var. *niveum* later renamed as *Fusarium oxysporum* f. *niveum* by Snyder and Hansen (1940). The fungus in Bombay is, therefore, *Fusarium oxysporum* f. *niveum*; whether it is a biologic form of the American pathogen or not, will have to be ascertained by cross-inoculation tests with both these fungi.

**HOST RANGE.** In order to study the host range of the fungus, 10 seeds of watermelon (*Citrullus vulgaris* L.), Muskmelon (*Cucurbita moschata* Duchesne), bitter gourd (*Momordica charantia* L.), 2 varieties of cucumber (*Cucumis sativus* L.), red pumpkin (*Cucurbita maxima* Duchesne), snake gourd (*Trichosanthes anguina* L.), bottle gourd (*Lagenaria bucantha* Rusby), Ridge gourd (*Luffa acutangula* Roxb.), and smooth gourd (*Luffa aegyptiaca* Mill.), were sown in soil infected with watermelon fungus and kept on the glasshouse benches. Watermelon sown in sterilized soil served as control. None of the other plants except watermelon showed wilting even after 1½ months. Only one plant of muskmelon died due to wilt as could be seen by the presence of fungus hyphae in the xylem vessels. Similar results were obtained in repeated trials, showing thereby that the fungus is highly pathogenic to watermelon and only slightly to musk melon.

**VARIETAL RESISTANCE.** As growing of wilt resistant varieties is the only solution for control of *Fusarium* wilts, the varieties Poona (local) Bombay (local), Kumpta (local), Yawal (Local), Muskati, Farukabadi, Faizabadi and Jaunpuri were tested for resistance to wilt in infested soil in a glass house at Poona but all were found to be susceptible.

#### SUMMARY.

Watermelon wilt was first noticed in India in January, 1955 in Dorli village of Thana district in a serious form.

Symptoms of the disease are described.

The pathogen was isolated, inoculation experiments carried out, pathogenicity proved and reisolation was done.

Measurements of micro and macro-conidia on different media are given.

The host range and varietal resistance are described.

The fungus is indistinguishable from *Fusarium oxysporum* f. *niveum* (E.F.S.) S. & H.

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# EFFECT OF DIFFERENT PHOSPHATES ON CHARACTERISTICS OF RHIZOBIUM OF GUAR (*CYAMOPSIS PSORALIOIDES*), IN DELHI SOIL

RAN BIR REWARI AND ABHISWAR SEN.

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Legumes have been known to respond very favourably to application of phosphates (Powers, 1942; Roberts and Oleson, 1944; Robinson, 1945). Very little information is, however, available as regards changes if any, imparted by fertilizers, particularly phosphates on the characteristics of rhizobium. It is known, however, that environmental conditions, climatic or those pertaining to soil, do effect at least the efficiency of a rhizobium. Rajagopalan (1938) observed that strains of ground nut (*Arachis hypogea*) rhizobium at Palacuppam had higher nitrogen fixing capacity than those isolated from Coimbatore. The former had also higher capacity of gum production, sensitivity to acidity and ferment power.

From the improvement of nodulation by phosphates in many legumes (Wilson, 1917; Thornton, 1929; Rogers and Sturkie, 1939) one would expect that phosphates would improve the efficiency of rhizobium. Truesdell (1917) showed that phosphates distinctly stimulated multiplication of root nodule organisms and were particularly conducive to the development of motile forms. Growth of rhizobium and fixation of nitrogen in the nodules of soybean was observed by Poschenrieder, Sammet and Fischer (1940) to increase with increased application of phosphate.

During the present investigations, guar was grown in Delhi soil with and without treatment of different phosphates and strains of rhizobium from a single multilobed nodule on the main root of a single healthy plant selected at random under each treatment. The characteristics and nitrogen-fixing capacities of the strains were determined. The object of the experiment was to see how far application of phosphates helped in the production of efficient strains.

**EXPERIMENTAL PROCEDURE.** Guar was grown in pots containing 30 lbs of Delhi soil with and without treatments of phosphates i.e. superphosphate, bonemeal, Singhbhum phosphate and Trichi nodules. The phosphates were added to the soil in the form of powder at the rate of 5 mg/ 100 gms of soil or 100 lbs of  $P_2O_5$  per acre. The plants were allowed to grow for about ten weeks after which a healthy plant under each treatment was selected at random and a healthy multilobed nodule on the main root of each plant was selected for isolation of the rhizobium

The nodules were detached from the root leaving a small portion of the root attached to them. They were then first washed with tap water

and then with distilled water and then surface sterilised with  $\text{HgCl}_2$  solution (1 : 1000) and alcohol. The sterilising liquids were then removed by repeated washings with sterile water and the nodules crushed under sterile water in a sterilised test tube with a sterilised rod. A loopful of the suspension was diluted with sterile water and a loopful of the diluted suspension plated in yeast extract mannite agar. Based on the similarities of the colony characteristics one or more single colony cultures was or were obtained. The isolates were plated again in Ashby's mannite agar containing Congo-red (1 : 20,000) and also on nitrate mannite agar whose pH was adjusted to 11, to test for the presence of the common contaminants like *Achromobacter* and *Phytomonas*.

Ultimately the number of strains obtained in pure cultures were: one from control, two from superphosphate, one from bonemeal, one from Singbhum phosphate and one from Trichi nodules.

TABLE I. Characteristics of the strains of the rhizobium of guar under treatments with different phosphates.

Treatment	Strains	Description of colonies used for isolation of the strains	Characteristics of the pure strains
Control	1	Spherical, round, entire, white, slimy and moist.	Unevenly stained rods, 1.2 $\mu$ - 4.0 $\mu$ Coccoids 0.7 $\mu$ - 1.5 $\mu$ diameter, motile with peritrichous flagella, gram negative.
Superphosphate	1	Round, entire, raised, white, little slimy, moist.	Unevenly stained rods 2.0 $\mu$ - 4.0 $\mu$ coccoid 0.5 $\mu$ to 1.5 $\mu$ diameter, motile with peritrichous flagella, gram negative.
	2	Irregular, undulated, raised, yellowish, not slimy, moist, opaque.	Coccoids 0.5 $\mu$ - 1.0 $\mu$ diameter motile with peritrichous flagella, gram negative.
Bonemeal	1	Spherical, entire convex, yellowish white, slimy moist, translucent.	Uniformly stained coccoids 0.5 $\mu$ - 1.0 $\mu$ motile with peritrichous flagella, gram negative.
Singbhum phosphate	1	Elliptical, entire, raised, white, granular, slimy, moist.	Uniformly stained small rods 0.5 $\mu$ - 2.0 $\mu$ and coccoids motile with peritrichous flagella, gram negative.
Trichi nodule	1	Elliptical, entire, raised, yellowish white, slimy and moist.	Uniformly stained rods 0.5 $\mu$ - 2.0 $\mu$ and coccoids, motile peritrichous flagella, gram negative.

RESULTS. The types of colonies on yeast extract mannite agar from which the strains had been isolated and some characteristics of the pure strains are given in Table 1.

FERMENTATION CHARACTERISTICS. Ashby's yeast water was used as basal medium. 1 percent solution of the following sugars in the above medium was prepared : dextrose, sucrose, lactose, maltose and galactose. One loopful of the culture of each strain was used as inoculum in each of the above solutions. Growth and gas formation in the media were examined after seven days.

NITROGEN FIXATION BY THE STRAINS. Nitrogen content of 8 weeks' old plants on sterile sand with and without inoculation with the different strains was determined. The plants were watered with sterile Crone's mineral solution during their growth.

FERMENTATION CHARACTERISTICS. The fermentation characteristics of the different strains are given in Table II.

TABLE II. Growth and change in reaction produced by strains of guar rhizobium in different sugars.

Sugars	Control	Superphosphate	Bone meal	Sing- bhum phosphate	Trichi nodule
	1	1	2	1	1
<i>Dextrose</i>					
pH	8.5	7.5	8.0	6.8	7.0
Growth	++	++	+++	++	++
Pellicle	TP	P	P	NP	NP
Uninoculated pH	7.0				
<i>Sucrose</i>					
pH	8.0	7.0	6.0	8.5	8.5
Growth	++	++	++	++	++
Pellicle	NP	NP	TP	NP	NP
Uninoculated pH	8.5				
<i>Lactose</i>					
pH	8.5	8.5	7.5	8.5	8.5
Growth	+	+	++	+	+
Pellicle	NP	NP	P	TP	NP
Uninoculated pH	7.0				
<i>Maltose</i>					
Growth	++	+	+	++	+
Pellicle	P	NP	NP	TP	TP
Uninoculated pH	7.5				
<i>Galactose</i>					
pH	8.0	6.0	6.5	7.0	6.5
Growth	+	+	+	+	++
Pellicle	TP	NP	NP	NP	TP
Uninoculated pH	6.0				

+ Slight growth  
 ++ Moderate growth  
 +++ Good growth

P Pellicle  
 NP No pellicle  
 TP Thin pellicle

It could be seen that differences were observed between the fermentation characteristics of the strains.

**CULTURAL CHARACTERISTICS.** All the strains grew well in soil extract mannite agar, Ashby's mannite agar and yeast extract mannite agar. Growth in the last medium was more rapid (24 hours) than the former (72 hours). The growth in slants was colourless, glistening, raised, slimy at the top and opaque milky white at the bottom.

**REACTION TO LITMUS MILK** Differences were also observed in their reaction to litmus milk. The observations are given in Table III.

TALBE III. Reactions of the strains of guar rhizobium in litmus milk after 4 weeks' growth.

Strains from treatments			Reaction	Serum zone
Control	1.	...	... Slightly alkaline	absent
Superphosphate	1.	...	... Acidic	present
	2.	...	... Alkaline	absent
Bonemeal	1.	...	... Acidic	present
Singbhum phosphate	1.	...	... Acidic	present
Trichi nodules	1.	...	... Acidic	present

**RELATIVE EFFICIENCY OF THE STRAINS.** The nitrogen content of the plants grown from sterilised seeds and from those inoculated with the different strains of the rhizobium are given in Table IV. The inoculated plants nodulated freely but since nodules were small they were not detached from the roots and nitrogen estimations in the roots were carried out along with the nodules.

**DISCUSSION OF RESULTS.** It can be observed from the data in the foregoing tables that the strains of guar rhizobium isolated from single nodules from plants with and without treatments of different phosphates are all motile and gram negative but they vary in size and sometimes in fermentation characteristics and in their reactions in litmus milk. From these variations it is not possible, however, to evaluate the exact effect of a particular phosphate on the rhizobium as the variations observed between themselves are also observed in strains isolated from plants under the treatment of the same phosphate, such as superphosphate.



TABLE IV. Nitrogen content of guar plants with and without inoculation with the different strains of rhizobium.

Inoculating Strain	Repl-ication	Dry wt. of plants (gm)			Nitrogen (%)		Total N- in plants per pot (mg)	Increase in N- over uninoculated (%)
		Shoot	Root	Total	Shoot	Root		
No inoculation	1	0.26	0.12	0.38	4.16	2.54	13.87	
	2	0.40	0.14	0.54	3.25	2.25	16.15	
	3	0.19	0.10	0.29	...	...	...	
					Average		15.01	
Control (1)	1	0.32	0.16	0.48	3.76	2.06	15.33	
	2	0.40	0.16	0.56	2.87	1.18	13.37	
	3	0.40	0.24	0.64	3.91	2.38	21.35	
					Average		16.68	11.14
Super-phosphate (1)	1	0.26	0.13	0.39	4.04	2.92	14.29	
	2	0.22	0.14	0.36	3.41	2.07	10.40	
	3	0.52	0.21	0.73	3.66	2.78	24.86	
					Average		16.52	10.06
(2)	1	0.38	0.21	0.59	4.76	2.74	23.84	
	2	0.86	0.32	1.18	3.40	2.68	37.72	
	3	0.53	0.15	0.68	3.67	3.00	23.95	
					Average		28.50	89.86
Bonemeal (1)	1	0.42	0.19	0.61	4.19	3.03	21.20	
	2	0.57	0.21	0.78	3.91	2.46	27.33	
	3	0.24	0.14	0.38	4.86	2.22	14.80	
					Average		21.11	40.63
Singbhum phosphate (1)	1	0.36	0.15	0.51	3.47	2.53	15.69	
	2	0.35	0.15	0.50	3.20	2.13	14.40	
	3	0.28	0.12	0.40	4.22	3.00	15.42	
					Average		15.17	1.06
Trichi nodule (1)	1	0.24	0.10	0.34	3.71	1.30	10.20	
	2	0.29	0.15	0.44	3.66	2.50	14.36	
	3	0.43	0.23	0.66	4.00	2.22	22.30	
					Average		15.62	4.06

From a comparison of the total amount of nitrogen contained in inoculated plants with that in plants raised under sterile conditions, (Table IV) it is found that plants inoculated with the strain superphosphate (2) gave the largest increase in the nitrogen content. The next higher increase

in nitrogen content occurs in the case of plants inoculated with bonemeal (1). The increase in nitrogen content of plants inoculated with superphosphate (1) is of the same order as that in the case of plants inoculated with a the strain from control. Strains from Singbhum phosphate (1) and Trichi nodules (1) have not given increase in nitrogen to any remarkable extent.

#### SUMMARY AND CONCLUSIONS.

During studies on rhizobium of guar raised with and without different phosphates it was observed that two strains differing distinctly from one another in their fermentation characteristics, reaction in litmus milk and in nitrogen fixing capacity could be obtained from a single nodule under the treatment of superphosphate. When guar was raised with other phosphates like bonemeal, Singbhum-phosphate and Trichinodule only one distinct strain could be obtained from a single nodule.

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## TAXONOMIC STUDIES OF UROMYCES ON INDIGOFEA SPECIES IN INDIA

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The rust, *Uromyces orientalis* Syd. was first recorded on *Indigofera linifolia* from India by Sydow and Butler (1907) and from Australia by Langdon and Herbert (1944). Holway (1901) and West (1939) have recorded another rust *Uromyces indigoferae* on many species of *Indigofera* from Gautemala, Salvador, Costa Rica and the U.S.A.

During the course of present studies, a rust on *I. tinctoria* was observed at Bapatla (Andhra Pradesh) and the causal organism was identified as a species of *Uromyces*. Since it appeared to be the first record of *Uromyces* on *I. tinctoria* in India, it was felt necessary to take up comparative morphological studies of the rust occurring on different *Indigofera* spp.

The type material of *U. orientalis* on *I. linifolia* and that of *U. indigoferae* on *I. mexicana* were obtained from the Herb. Crypt. Ind. Orient and from Dr. G. B. Cummins of Purdue University (U.S.A.), respectively.

Important characters of taxonomic value such as size of the uredo and teleutospores, thickness of the uredospore wall, epispore, endospore and apex of the teleutospores were recorded on individual material. The data are set in Table I.

From the data presented in the Table it will be seen that there does not appear to be any appreciable difference in the measurements of the spores of the rusts on different species of *Indigofera* except that the uredospores of the rust on *I. linifolia* are slightly smaller in length than the rest. Even as regards the colour and shape of the spores, all of them appear more or less alike.

Sydow and Butler (1907), while describing *U. orientalis*, stated that it is distinguished from *U. indigoferae* by its smaller uredospores. Thus the criterion for speciation of *U. orientalis* and *U. indigoferae* are mainly based on the size of the uredospores. However, the present studies do not reveal any such significant differences of taxonomic value between *U. indigoferae* and *U. orientalis*. Even the material collected on *I. tinctoria* at Bapatla was found to be much similar to the rest and it was felt that the differences exhibited by uredospores from different hosts do not warrant the separation of the two species. It was primarily to remove this confusion over nomenclature of the rust, that comparative morphological and pathological studies were undertaken. In addition to morphological characters, as stated above, there are no appreciable differences in the mode of germination of uredo- and teleutospores, the formation and measurements of promycelia and sporidia, longevity of spores in storage under

TABLE. 1 Comparative measurements of the uredo and teleutospores of the rust on different species of Indigofera.

S. No.	Host	Uredospores		Teleutospores		
		Size	Thickness of the wall	Size	Epispore	Endospore apex
1.	<i>Indigofera linifolia</i> (Collected from Delhi area).	20 $\mu$ x 18 $\mu$ .	1.5-2.0 $\mu$	27 $\mu$ x 22.5 $\mu$	1.8-3.6 $\mu$	14.6-21.9 $\mu$ 3.6-9.1 $\mu$
		(18-23 $\mu$ x 14.6-22 $\mu$ )		(22-32 $\mu$ x 18-25 $\mu$ .)		
2.	<i>I. glandulosa</i>	21.5 $\mu$ x 18.5 $\mu$ .)	"	"	"	"
		(18-25.6 $\mu$ x 14.5-22 $\mu$ )	"	"	"	"
3.	<i>I. cordifolia</i>	21.5 $\mu$ x 18.5 $\mu$ .)	"	"	"	"
		(18-25.6 $\mu$ x 14.5-22 $\mu$ )	"	"	"	"
4.	<i>I. linifolia</i> (Type specimen)	20.8 $\mu$ x 18.5 $\mu$	"	"	"	"
		(18-23 $\mu$ x 14.6-22 $\mu$ )	"	"	"	"
5.	<i>I. mexicana</i> (Type specimen)	21.5 $\mu$ x 18.4 $\mu$	"	22-29 $\mu$ x 17-23 $\mu$ *	1.8-3.5 $\mu$ *	15.2-20.0 $\mu$ * 7-10 $\mu$ *
		(18-25.6 $\mu$ x 14.5-22 $\mu$ )	"	"	"	"
6.	<i>I. tinctoria</i> (Collected from Bapatla)	21.5 $\mu$ x 18.4 $\mu$	"	27 $\mu$ x 22 $\mu$	1.8-3.6 $\mu$	14.6-21.9 $\mu$ 3.6-10 $\mu$
		(18-25.6 $\mu$ x 14.5-22 $\mu$ )	"	(22-32 $\mu$ x 18.25 $\mu$ )	"	"

N.B. \* Measurements are given by Arthur (1934).



different conditions and other minor characters of the rust samples from *I. linifolia* and *I. tinctoria*. These studies revealed that the two samples are almost alike and hence it is suggested that they may be treated as one species. Since *U. indigoferae* Diet. & Holw. has been described earlier by Holway (1901), it would be proper to call the rust as *U. indigoferae* Diet. & Holw. (Syn. *U. orientalis* Syd.) which is considered to be the correct name of the rust on *I. linifolia* and *tinctoria*.

#### SUMMARY.

A rust collection from Bapatla (Andhra State) on *Indigofera tinctoria* was identified as *Uromyces indigoferae*. Comparative morphological studies have shown that there is very little difference between *U. orientalis* on *I. linifolia* and this species. The measurements of the type specimens of *Uromyces orientalis* (on *I. linifolia*, *I. cordifolia* and *I. glandulosa*) and *U. indigoferae* (*I. mexicana*) revealed no significant differences to warrant their separation into two species. It is, therefore, proposed to call the fungus *U. indigoferae* Diet. & Holw. (Syn. *U. orientalis* Syd.)

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The authors also wish to express their thanks to Dr. G. B. Cummin of Purdue University, for sparing the type specimen of *U. indigoferae* Diet. and Holw.

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**A TRICOTHECIUM SP. (CEPHALOTHECIUM SP.) AS A HYPER  
PARASITE ON PUCCINIA GRAMINIS TRITICI (PERS.) ERIKSS.  
AND HENN.**

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Occurrence of hyperparasites on wheat rusts has been reported by many workers. In India, Prasada (1948) has recorded *Darluca filum* on *Puccinia graminis tritici* and *Puccinia triticina*. During the routine glasshouse work on the maintenance of physiologic races of wheat rusts at Delhi in November, 1955 it was observed that sometimes when the humidity in the glasshouse was very high (60 to 90 percent) the uredopustules of black rust of wheat were covered with a reddish white, fluffy mycelium (Fig. 1). The fungus was first isolated in pure culture during December, 1955 and was indentified as *Trichothecium roseum* Link. The fluffy growth on uredopustules of black rust was again observed during October, 1956. When isolations were made from the affected pustules of the leaves, *Trichothecium roseum* and another unidentified fungus were isolated. As *Trichothecium roseum* was consistently found occurring on the uredopustules for two years successively the study of association of this fungus with *Puccinia graminis tritici* was taken up.

On Potato-Dextrose-Agar, the mycelium is widespread forming a reddish-white tuft consisting of branched septate hyphae measuring 3.6 to 7.2  $\mu$  in thickness. The conidia are normally bicelled, pear-shaped, pinkish in colour measuring 7.2-20.7 x 5.4-9  $\mu$  (with an average of 17.5 x 7.8  $\mu$ ) and are found in clusters on the top of conidiophores.

To study the relationship of this fungus with the black rust of wheat a suspension of *Trichothecium roseum* spores was made in sterile distilled water and was sprayed by an atomizer on wheat seedlings in which the first leaves had unfolded. In another treatment, plants of the same age were taken and slightly injured before spraying the spore suspension. In the third treatment, plants showing well-developed uredopustules of black rust were selected and sprayed with spore suspension. Wheat seedlings with uredopustules were sprayed with sterile distilled water and kept as control. All the 4 sets of pots were kept at high humidity (about 80%) and at a temperature range of 77° to 90°F, under identical conditions. It was observed that no fungal growth appeared in treatments 1, 2 and 4 but in the third treatment a fluffy growth of mycelium appeared on uredopustules within 5-7 days. The mycelium, however, did not develop on healthy tissues.

When the uredospores of black rust mixed with the spores of *Trichothecium roseum* were kept for germination, the uredospores did not show any germination whereas there was about 100% germination in the

control. When wheat seedlings were inoculated with a mixture of black rust uredospores and spores of *Trichothecium roseum*, practically no rust pustule was produced, indicating that the fungus adversely affected the germination of the rust spores.

Text Fig. 1

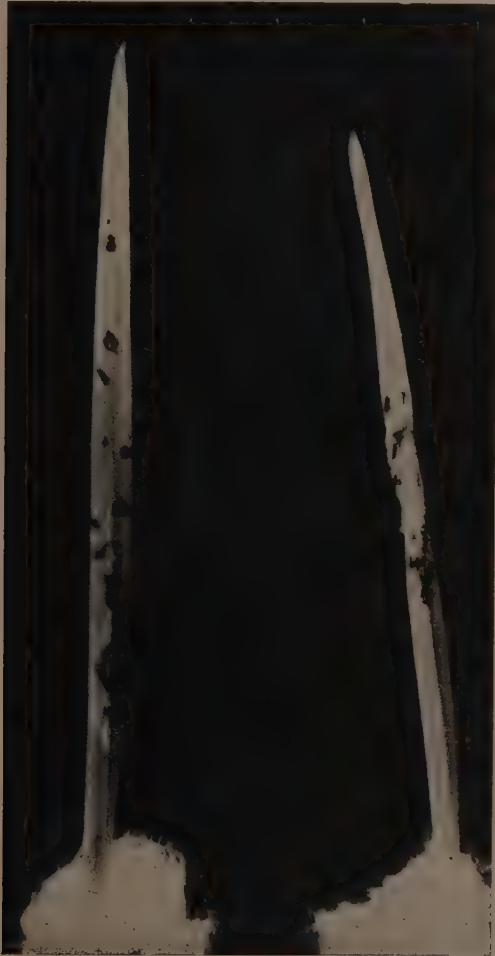


Fig. 1. Wheat leaf on the left showing healthy uredopustules of black rust and the one on right shows mycelium of *Trichothecium roseum* developing on the uredopustules

Table 1: Percentage germination of uredospores of *Puccinia graminis tritici* in various concentrations of culture filtrate of *Trichothecium roseum*.

	Percent strength of culture filtrate								Sterile *PDY Broth		Sterile Distilled water
	1	5	10	20	30	40	50	80	Full strength		
Percentage germination	33.3	10	6.4	2.35	0	0	0	0	0	97	99
Length of germ tube ( $\mu$ )	74.41	17.78	14.88	14.52	0	0	0	0	0	83.85	158.9
Breadth of germ tube ( $\mu$ )	5.44	5.44	5.44	5.44	0	0	0	0	0	5.44	3.63

\* PDY = Potato Dextrose Yeast-extract broth

It has been recorded by Gupta and Price (1952), Bawden and Freeman (1952) that the growth products of *Trichothecium roseum* Link. inhibit the development of viruses in plants. In order to find out whether this fungus produces any inhibitory substance which renders the uredospores incapable of germination the organism was cultivated on Potato-Dextrose-Yeast-extract broth (500 c.c., of fifty percent potato extract, 20 gms. Dextrose and 10 gms. Yeast extract, in one litre. Fifty c.c. of the medium was taken in 250 c.c. Erlen Mayer flasks). After eight days growth of the organism the culture filtrate was taken and used for testing the germination of the uredospores. The results of the tests are given in Table. I.

It is evident from the data presented that the organism produces certain metabolic products which inhibit the germination of uredospores of black rust of wheat. Uredospores sown as control in the Potato-Dextrose-Yeast-extract broth generally developed stouter and slightly shorter germ-tubes than those in distilled water.

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# FRUIT ROT OF *CITRULLUS VULGARIS* VAR. *FISTULOSUS* L.

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**INTRODUCTION.** Tinda (*Citrullus vulgaris* var. *fistulosus* L.) is a climbing cucurbitaceous herb, cultivated for the sake of its fruit, which is used as a delicious summer vegetable. It is grown extensively in Rajasthan, Uttar Pradesh, Madhya Pradesh and Punjab for its cash return to the cultivator. The plantations are generally located near big cities.

A rotting of tinda fruits responsible for considerable damage to vegetable sellers was noticed in Kota (Rajasthan) market. In the present paper a brief account of the disease and the identity of the causal organism have been reported and control measures suggested.

**SYMPTOMS.** In the beginning, small yellowish spots appear on the surface of the fruit; later in damp weather, skin around the spots turns light brown and is covered with a white aerial growth of the fungus, and finally the fruit becomes soft and rots. On cutting, the rotted area of fruit is found to be of light brown colour from within and gives foul odour.

TABLE. I. Macroscopic growth characters of the fungus causing fruit rot of tinda on different media.

No.	Medium	Colour of aerial mycelium	Colour of the growth substrate	Colour of the medium
1.	Potato dextrose agar 2%	Mycelium moderate white in colour	Ivory yellow	nil
2.	Potato dextrose agar 5%	Mycelium moderate white in colour	nil	nil.
3.	Maize meal agar	Mycelium fluffy white in colour	Ivory yellow	Ivory yellow.
4.	Czapek dextrose agar.	Mycelium fluffy white in colour	Dark purple	Purple
5.	Brown's starch agar.	Mycelium moderate white in colour.	nil	nil.
6.	Potato plug.	Mycelium white in colour	Ivory yellow	nil
7.	Steamed rice.	Mycelium white & rosy at places.	Dark purple	nil

**MORPHOLOGICAL CHARACTERS.** The fungus *Fusarium* was isolated from the affected fruits on plating. The culture was purified by single spore technique. The growth characters and sporulation of the organism were studied in 10 cm. plates on different media. Uniform discs of four days old mycelium, 2 mm. in diameter cut from the periphery in water agar plates were used as inoculum and plates were kept at room temperature (28°C to 30°C) and the observations recorded after 20 days. The macroscopic growth characters are presented in Table 1.

**SPORULATION.** The microconidia were ellipsoid to ovoid in shape, hyaline, and constituted the majority; the macroconidia one to three septate, with delicate walls and sickle shaped; the conidiophores simple. The size of conidia and their relative production studied on different media is given in Table II.

TABLE No. II. Size of different conidia and their relative production observed on different media after 20 days of inoculation with *tinda* fruit rot fungus.

S. No.	Medium	Type of conidia	Production in percent-age	Range in size in microns.	Average size in microns.
1.	Potato dextrose agar 2%	0 Septate	89.4	6.4-11.2 x 1.6-3.2	8.6 x 2.9
		1 Septate	7.4	12.8-19.2 x 2.4-3.2	15.8 x 2.6
		3 Septate	3.2	25.6-38.4 x 2.4-4.8	32.3 x 3.6
2.	Browns starch medium	0 Septate	76.3	8.0-12.8 x 2.4-3.2	10.2 x 2.7
		1 Septate	13.0	12.8-24.0 x 2.4-3.2	19.5 x 2.9
		2 Septate	1.3	19.2-22.4 x 2.4-3.2	21.3 x 2.9
		3 Septate	9.4	25.6-41.6 x 3.2-4.8	34.9 x 3.9
3.	Czapek dextrose agar	0 Septate	93.0	6.4-11.2 x 1.6-3.2	9.0 x 2.5
		1 Septate	5.7	11.2-19.2 x 2.4-3.2	15.0 x 2.7
		3 Septate	1.3	22.4-38.4 x 2.4-4.0	31.2 x 3.5
4.	Steamed rice	0 Septate	92.7	6.4-11.2 x 1.6-3.2	8.5 x 2.7
		1 Septate	5.1	12.8-19.2 x 2.4-3.2	16.3 x 2.9
		3 Septate	2.2	25.6-40.0 x 2.4-4.0	32.3 x 3.8

Chlamydospores terminal and intercalary, spherical to pear shaped, smooth, hyaline, 1 to 2 celled, measuring 8.0-11.2 x 7.2-8.8 microns; sporodochia, sclerotia, and pionnotes absent.

**IDENTIFICATION OF THE CAUSAL ORGANISM.** Stroma pale to purple non-erumpent; white aerial mycelium; microconidia ellipsoid to ovoid, constituting about 90% of the conidia produced; macroconidia few, one to three septate with delicate walls, sickle shaped; conidiophores simple;

chlamydospores terminal and intercalary, one to two celled, spherical to pear shaped, and smooth; sporodochia, pionnotes and sclerotia absent.

On the basis of the above characters the fungus is identified as *Fusarium oxysporum* (Schl. ex. Fr.) emend. Snyd. et. Hans.

Synonym. *Fusarium orthoceras* App. and Wr. (Baari).

**PATHOGENICITY TEST.** Tests to determine pathogenicity of the fungus were carried out by means of a series of inoculations. Selected fruits of the same variety, size, and maturity were surface sterilised by dipping them in 2/1,000 potassium permanganate solution for 20 minutes, then washed with sterile distilled water and surface wiped with absolute alcohol. Spray of conidial suspension from one week old culture on 2% potato dextrose agar was used for inoculation, while sterile distilled water was sprayed in the control. The following four treatments were tried:

1. Fruits punctured with needle and heavy spore suspension sprayed.
2. Fruits unpunctured and heavy spore suspension sprayed.
3. Fruits punctured and sterile distilled water sprayed.
4. Fruits unpunctured and sterile distilled water sprayed.

After treatment all the fruits were kept in moist chamber at room temperature of 28°C to 30°C. Within 48 hours, typical diseased spots were noticeable on the punctured inoculated fruits. These spots continued to increase in size and there developed the white aerial growth of the fungus within 4 days. Within 8 days, the fruits had entirely rotted. There was no sign of any infection on the uninjured and control fruits by that time. The results of inoculation tests are recorded in table III.

TABLE III. Results of pathogenicity tests of *tinda* fruit rot fungus.

No.	Treatment of fruits	Number of inoculations.	Number of fruits infected.
1.	Punctured with needle and heavy spore suspension sprayed. ...	6	6
2.	Unpunctured and heavy spores suspension sprayed. ...	6	0
3.	Punctured and sterile distilled water sprayed. ...	4	0
4.	Unpunctured and sterile distilled water sprayed. ...	4	0

It is clear from the Table III that the fungus is a weak parasite and cannot enter through uninjured, healthy skin of the fruit.

METHODS OF CONTROL. Since the fungus is a wound parasite, all attempts should be made to avoid mechanical injuries. The disease incidence can very well be minimised by the grower and the dealer if following preventive methods are followed:

1. Harvesting under good conditions.
2. Exclusion of badly diseased produce from any consignment.
3. Careful picking and careful handling and packing to reduce the possibility of mechanical injury.
4. Washing the fruits only when really necessary.
5. Quick transport and sale of the produce.

ACKNOWLEDGEMENT. Our sincere thanks are due to Shri Samarth Raj, Director of Agriculture, Rajasthan, for encouragement and providing the facilities.

#### SUMMARY

The fungus causing fruit rot of *tinda* in storage in Kota is identified as *Fusarium oxysporum* (Schl. ex. Fr.) emend. Snyder et Hans.

The morphology of the fungus is described in detail.

Pathogenicity of the organism has been established. The inoculation experiments showed that the fungus cannot enter through uninjured skin of the fruit.

The fungus is a saprophyte and the spores float in the air and if they happen to settle down on injured surfaces of the *tinda* fruit, they cause rotting.

The effective method of control consists in careful handling, so as to avoid injuries to the fruit.

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## THE INDIAN SPECIES OF *MERULIUS*

B. K. BAKSHI AND BALWANT SINGH

(Accepted for publication March 18, 1958)

The four species of *Merulius* recorded in Butler and Bisby (1931) are *M. lignosus* on dead wood from the E. Himalayas (Darjeeling); *M. similis* on exposed bamboo roots from the plains of W. Bengal; *M. pseudolachrymans* on tree roots, in Saharanpur, Uttar Pradesh; and *M. corium*, whose report is not indefinite. *M. similis*, which is described in culture (Banerjee and Bakshi, 1945), is common in the plains of Bengal during the monsoons as large, yellow, bracket-shaped sporophores on the bases of living bamboo or their stumps, in the wood of which the fungus causes white fibrous rot. Recently *M. tremellosus* is described from sporophore and culture (Bagchee Puri and Bakshi, 1954). The fungus is common on dead oaks in the W. Himalayas. Bagchee (1954) discovered *M. lacrymans* on felled spruce in the open forests and also on worked wood in houses in the temperate regions of W. Himalayas. The Indian specimen has been described from sporophore but not in culture. Recently, Bakshi, Singh and Choudhury (1958) have identified *M. himantioides* growing on soft-woods in the temperate Himalayas. The 7 species listed above are all those recorded in India. The paucity in the number and records of species under *Merulius* is apparently due to inadequate attention in the study of wood-destroying fungi in India. In this paper, 3 species of *Merulius* are described, of which *M. aureus* and *M. confluens* are new records in India, while *M. corium* is imperfectly known. Annotated account of *M. tremellosus* is also given. *M. confluens*, *M. corium* and *M. tremellosus* are widely distributed in the world (Burt, 1917; Rea, 1922; Cunningham, 1950), while *M. aureus* was so far recorded only from Europe and N. America (Burt, 1917; Rea, 1922).

1. *Merulius aureus* Fr. Fructifications annual, effuso-reflexed with narrow margin upto 2 mm. wide, sometimes in small circular patches, soft but crustaceous when dried, 0.5 - 4 cm. long 0.5 - 3 cm. wide and .03 - 0.6 cm. thick, with pale orange rhizomorphic strands in the crevices of wood. Upper surface 'buff yellow'† changing to 'yellow ochre'. Hymenium yellow-orange, 'ochraceous orange' when fresh changing to 'russet' and 'cinnamon brown' later, longitudinal folds more prominent than the transverse ones to give gill-like appearance but hymenium definitely poroid (Pl. I, fig. 1). Context upto 2 mm. thick in resupinate forms, upto 5 mm. in reflexed portions, composed of loosely interwoven, thick walled, clamped, branched hyphae (Text-fig. 1a), 3 - 5  $\mu$  broad. Basidia clavate (Text-fig. 1b), 10 - 18 x 2.5 - 4  $\mu$ . Basidiospores nearly hyaline to light pale, becoming darker in old specimens, cylindrical to oval, thin-walled, 1 - 2 guttulate (Text-fig. 1c), 2.5 - 4.6 x 1.5 - 1.8  $\mu$ .

On logs of *Picea morinda*, *Abies pindrow*, *Pinus excelsa*, *Cedrus deodara*, Western Himalayas. The fungus causes brown cuboidal rot in the wood.

† Colours within commas are described from Ridgway (1912)



Dr. R.W.G. Dennis remarks on a specimen sent to him that "It may be a pileate state of *M. aureus* but is considerably thicker than other collections so referred". Dr. L. Harmsen considers it to be *M. aureus*, 'even if the spores seem to be rather small'. We have collected more specimens subsequently in which the fruit body is thinner and spores longer than the specimen referred to them, and is therefore typical.

**GROWTH CHARACTERS.** Growth\* 6-8 mm. Advancing zone even, appressed. Mat appressed to cottony (Pl. I, fig. 2), 'cream buff' to 'colonial buff'. Reverse dark brown with concentric zones in 2-3 weeks. Odour none. On gallic acid agar, diffusion zones absent, growth 6-8 mm., on tannic acid agar, diffusion zones moderate, growth nil. Colony 8 mm. on former and nil on latter. On malt agar containing gentian violet, medium unbleached, growth slow.

**HYPHAL CHARACTERS.** Advancing hyphae nearly hyaline or pale coloured, thin-walled or slightly thick-walled, branched with clamps (Text-fig. 1d), 1.5-4  $\mu$  broad. Aerial and submerged mycelia, same as above.

Text Fig. 1 a - d



1. *M. aureus*, a. context hyphae; b. basidia, c. basidiospores; d. culture hyphae.

The fungus is readily distinguished by the colour of the fruit body, folds in the hymenium which are prominent in longitudinal direction more than in transverse ones, clamped hyphae both in sporophore and culture, slow growth in culture which shows concentric zones on the reverse of plates.

2. *Merulius confluens* Schw. Fructifications annual, coriaceous, broadly effused (Pl. I, fig. 3) 10 cms. long, 1-2 cm. wide, 0.4-0.7 mm. thick with reflexed inturned margins upto 5 mm. Upper surface whitish, finely tomentose, lightly concentrically zoned. Hymenium flesh pink ('pinkish cinnamon', 'pears brown'), continuous, with shallow reticulate pores, 2-3 per mm. Context white, spongy, composed of loosely interwoven, thin-walled (Text-fig. 2a) or thick-walled, septate hyphae, usually incrustated towards hymenium (Text-fig. 2c), branched, rarely with clamps (Text-fig. 2b), 3-5.5  $\mu$  broad, Irregularly globose or elliptical bodies

\* Growth in all cases refers to radial growth in 7 days in malt agar at 25°C in dark,

present. Basidia clavate (Text-fig. 2d), persistent,  $16 - 20 \times 4 - 6 \mu$ . Basidiospores hyaline, thin-walled, elliptic, (Text-fig. 2e),  $4 - 5.8 \times 2.5 - 3 \mu$ .

On dying branches of cultivated rose, *Shorea robusta* and *Castanea sativa*, Dehra Dun, and *Quercus semicarpifolia*, Chakrata, U.P.

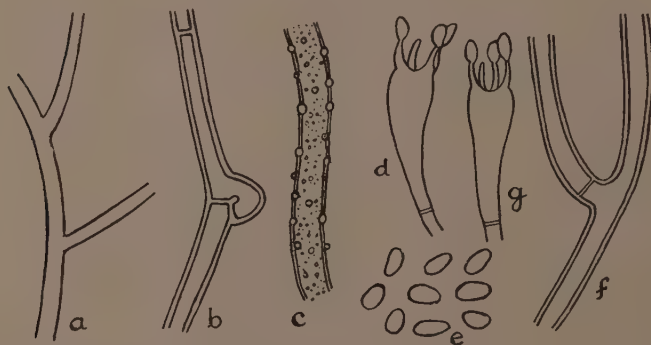
Two American collections of *M. confluens*, one collected by J.E. Bier and determined by H. S. Jackson and another collected by Irene Mounce, are deposited in our herbarium. These agree with the Indian specimens of the fungus.

Identification confirmed by Dr. L. Harmsen.

**GROWTH CHARACTERS.** Growth 2.8 cm. Mat even, hyaline, appressed (Pl. I, fig. 4). Pored fruiting surfaces 'flesh pink', develop mostly along the side of Petri dishes in 2 weeks. Reverse completely bleached. Odour fruity. On gallic acid agar, diffusion zones absent, growth 1.8 cm., on tannic acid agar diffusion zone strong, growth trace. On malt agar containing g-ation violet, medium partially bleached, growth-moderate.

**HYPHAL CHARACTERS.** Aerial mycelium: hyphae hyalin, thin-walled or slightly thick-walled (Text-fig. 2f), branched with simple septa,  $2 - 4 \mu$  wide. Submerged hyphae hyaline, thin-walled,  $3 - 6 \mu$  broad. Poroid fruit body in culture develop clavate basidia (Text-fig. 2g),  $14 - 20 \times 4 - 6 \mu$  bearing basidiospores, hyaline, ellipsoid,  $4 - 4.5 \times 2.2 \mu$ .

Text Fig. 2 a - f



2. *M. confluens* a. context hypha, thin-walled; b. context hypha, thick-walled; c. context hypha; incrustated; d. basidia; e. basidiospores f. culture hyphae; g. basidia in culture.

3. *Merulius corium* (Per.) Fr. Fructification annual, coriaceous, broadly effused (Pl. I, fig. 5), 30 cm. long, 2 - 3 cm. wide, 0.5 - 0.7 mm. thick, reflexed at margin upto 3 mm. Upper surface whitish to cream colour, free margin inturned, finely tomentose, sometimes concentrically zoned. Hymenium cream when fresh, drying 'flesh colour' to 'salmon orange', shining, continuous with shallow reticulations, pores 1 - 4 per mm. Context white, spongy, 0.5 - 0.6 mm. thick composed of loosely

interwoven hyphae, thick-walled, simple septate, branched (Text. fig. 3a) 3 - 6  $\mu$  broad, incrustated hyphae sometimes present. Basidia clavate (Text-fig. 3b), persistent, 15 - 24 x 4 - 6  $\mu$ . Basidiospores hyaline, thin-walled, elliptic, (Text-fig. 3c), 6 - 7.5 x 3 - 3.5  $\mu$ .

On dead branches of *Carissa opaca* and dead twigs of *Lantana camara*, Dehra Dun.

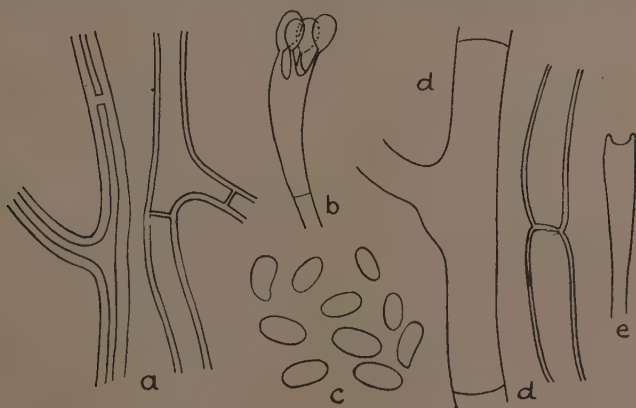
A specimen of *M. corium* in our herbarium collected in Germany and determined by H. Sydow agrees with the Indian specimen of the fungus.

Identification confirmed by Dr. L. Harmsen.

**GROWTH CHARACTERS.** Growth 2.8 cm. Mat hyaline, even, appressed to downy, hyphae condensing at places into small areas (Pl. I fig. 6) in 1 - 2 weeks with a tendency to form small fruitbodies along the side of the Petri dishes in 2 weeks. Reverse completely bleached. Odour strong fruity. On gallic or tannic acid agars, diffusion zones absent, growth 2 cm. on former, nil on latter. On malt agar containing gentian violet medium partially bleached, growth moderate.

**HYPHAL CHARACTERS.** Aerial mycelium : hyphae hyaline, thin-walled, branched with simple septa (Text-fig. 3d), very rarely with clamps, 2 - 7  $\mu$  broad. Submerged mycelium, same as above. No poroid fruit body is distinguished but along the glass surface, basidia develop which are mostly immature (Text-fig. 3e).

Text Fig. 3 a - e



3. *M. corium*, a. context hyphae; b. basidia; c. basidiospores; d. culture hyphae; e. basidia, immature.

## Plate 1



*Merulius aureus* Fig. 1- Sporophores on wood of *Pinus excelsa* ( $\times 1$ ). Fig. 2. Culture, 6 weeks, old ( $\times 2.3$ ).

*Merulius confluens*. Fig. 3 Sporophore on dead twig of *Castanea sativa* ( $\times 2$ ). Fig. 4 Culture, 2 weeks old ( $\times 3.7$ ).

*Merulius corium*. Fig. 5 Sporophore on dead wood of *Carissa opaca* ( $\times 1.5$ ). Fig. 6 Culture, 2 weeks old ( $\times 3.7$ ).

The two closely allied species *M. confluens* and *M. corium*, may be distinguished as follows. The hymenium in the former is generally darker than in the latter. In *M. confluens*, the walls of context hyphae are thinner and also, the basidiospores are smaller than those in *M. corium*. In culture, small poroid fruiting bodies, which develop in *M. confluens*, are absent in *M. corium*. *M. confluens* gives oxidase reaction on tannic acid agar but not on gallic acid medium; while *M. corium* gives no reaction on either.

4. *Merulius tremellosus* (Schräd.) Fr. *M. tremellosus* is very common mostly on oaks on which, the fungus in Western Himalayas is associated with white pocket rot. It is also collected on *Betula alnoides* and *Cedrus*

*deodara*. The basidiospores are hyaline, allantoid, guttulate (Text-fig. 4),  $2.9 - 3.2 \times 0.8 - 1 \mu$ .

Text Fig. 4



*M. tremellosus*, basidiosporas. (ali  $\times 1250$ ).

Among the collections of *M. aureus* studied, a few were made by Dr. K. Bagchee. Our grateful thanks are due to him. We are also thankful to Dr. R. W. G. Dennis and to Dr. L. Harmsen for advice on the identity of *Merulius* species described in this paper.

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## MERCULINE - A PROMISING SOIL FUNGICIDE

P. GOVINDA RAO and J. SUBBAIAH

(Accepted for publication March 15, 1958)

**INTRODUCTION.** Wilts in crop plants caused by the genera of *Fusarium*, *Sclerotium*, *Rhizoctonia*, *Pythium* etc. are quite a common occurrence in the Andhra State. The Panama wilt in banana, the *Pythium* rhizome rot in turmeric, the *Rhizoctonia* wilt in groundnut, the *Fusarium* and *Sclerotium* wilts in chillies and the *Sclerotium* wilt in betelvine are some of the examples. A good proprietary soil fungicide to solve the various wilt problems of the State was a long felt need. There are a number of proprietary fungicides in the market and some of them have been tested for their efficacy in controlling "damping off" and "wilt" diseases so that suitable ones could be recommended to the cultivators.

**MATERIAL AND METHODS.** Zentmyer (1955) has evolved a laboratory method of testing soil fungicides. The same method was adopted by the authors with very slight modifications for screening some of the promising fungicides. The fungicides selected were Merculine (an organic mercurial fungicide containing 10% Phenyl-mercurysalicylate), Cupravit (a copper fungicide containing 50% elemental copper as copper oxychloride) and Fungimar (another copper fungicide containing 50% elemental copper as cuprous oxide). The test organism selected was *Sclerotium rolfsii* (Sacc.) causing the wilt in chillies. The fungus was brought into pure culture from sclerotia and, its pathogenicity was tested on the susceptible hosts.

The typical sandy soil of Bapatla was air dried, sifted through a 20 mesh per inch sieve and autoclaved for 45 minutes at a pressure of 15 lb. per sq. inch in 1,000 c.c. flasks (Pyrex). For testing the suitability of the fungicides as a soil drench 1" length of soil is placed in sterile 2.5" x 1.0" specimen tubes. *Sclerotium rolfsii* was grown on oatmeal agar in Petri dishes and a disc of 10 m.m. diameter was cut with a sterile cork borer from the outer margin of the culture and was placed on the 1" column of the soil in the specimen tube. The disc was then covered with 1" of the same soil. 5 c.c. of the fungicidal solution under test was then applied to the surface of the soil in the tube. The tube was then incubated at the room temperature for 24 hours. After 24 hours the contents of the tube were emptied into a perforated metal strainer. The fungal material was picked out with a sterile forceps, moisture was removed by keeping on clean blotting paper and was placed in Petri dishes having oats agar medium for determining the viability of the fungus. The tests were conducted in triplicate. Controls were maintained in all cases and the results were recorded merely as presence or absence of growth of the fungus.

The same tests were repeated using the typical black soil of the chilli growing areas of Guntur District. In order to study the influence of the

contact period between the fungal discs in the soil columns and the fungicidal dilutions, another series of tests were conducted when the fungal discs were kept in contact with the fungicidal solutions for varying periods of 24, 48, and 72 hours and later incubated on oats agar medium in petri-dishes.

The three fungicides Merculine, Cupravit and Fungimar were also tried in field experiment for the control of the same pathogen. The test concentration of Merculine which was found to be fungicidal to *S. rolfii* in the laboratory experiments was utilized for the trial of the same in the field.

#### *Experimental results:—*

**LABORATORY TESTS:** Merculine proved highly fungicidal at the rate of 1 and 2 ozs. in 5 gallons. Both Cupravit and Fungimar at the doses tried were neither fungicidal nor fungistatic. With these two fungicides there was very slight initial cessation of growth of the fungal discs but the fungus overgrew the plates after 24 hours and then the growth was on a par with the control plates. The results obtained in the two types of soil were identical.

At 24, 48 or 72 hours of exposure of fungal discs to the fungicidal solutions, Cupravit or Fungimar at  $\frac{1}{4}$  oz or  $1\frac{1}{2}$  oz per gallon did not exert any fungicidal action. Merculins at 1 oz/5 gallons proved fungicidal at the three incubation periods while  $\frac{1}{2}$  oz/5 gallons proved fungicidal only at 48 or 72 hours and was fungistatic at 24 hours period. The results obtained are identical in either of the soil types.

**FIELD TESTS:** During May 1955 severe outbreak of Sclerotial wilt of chillies was reported from Yazali in the Guntur District, where the crop is raised in sandy soil. There was sudden wilting of four months' old plants in full fruit, with disease incidence up to 25%. Plots were selected from the worst affected chilly gardens and plants showing initial signs of wilting and completely wilted ones were removed prior to the starting of the fungicidal treatments. The fungicides Merculine @ 1 oz./5 gallons, Cupravit and Fungimar (at  $\frac{3}{4}$  oz/1 gl. were applied as drenches around the base of each plant. One gallon of fungicide solution was used for every 20 plants. Regular watering was taken up only 24 hours after the fungicidal drenching. Two drenches were given at 10 day intervals in the first trial and only one in the case of the second trial. The wilted plants were recorded periodically and the observations were continued for over two months. The results were evaluated on the basis of percentage of wilted plants. The data is given in Table I.

From a perusal of data in Table I it is evident that Merculine is very effective as a soil drench, when applied twice at 10 day intervals at 1 oz. in 5 gallons of water. Even when only one drench is given the wilt incidence has not gone beyond 2% while in the control it is 20%. Cupravit and Fungimar exercised only partial or no control of wilt giving 11.11% and 18.18% of wilt incidence respectively.

TABLE I: Effect of fungicides on wilt incidence

No.	Name of the Fungicide and dose used.	No. of plants treated	No. of plants wilted	% of wilted plants.
<i>Ist Trial:</i>				
1.	Merculine @ 1 oz./5 gl.	68	nil	0. 0
2.	Cupravit ,, $\frac{3}{4}$ oz./1 gl.	54	6	11.11
3.	Fungimar ,, $\frac{3}{4}$ oz./1 gl.	55	10	18.18
4.	Control.	76	23	30.26
<i>IInd Trial.</i>				
1.	Merculine ,, 1 oz./5 gl.	150	3	2. 0
2.	Control.	150	30	20. 0

## SUMMARY

Three fungicides Merculine, Cupravit and Fungimar were tested for their efficacy for the control of *Sclerotium rolfsii* Sacc. causing wilt in chillies in the laboratory and in the field as soil drenches. In the laboratory tests and also in the field trials Merculine at 1 oz. in 5 gallons of water gave effective control of the disease while the other two did not.

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## SOME NEW HOSTS OF RUSTS FROM INDIA

H. D. DUBEY

(Accepted for publication March 15, 1958)

**INTRODUCTION.** The author in course of his regular collection trips in the neighbourhood of the Deochanda Experiment Station, Hazaribagh (Altitude - 2,000'; average rainfall - 50") collected a number of plants infected with rusts. Of the 30 collections of rusts, the following four were particularly interesting as two of them (Nos. 1 and 2) were records on new host genera, and the other two (Nos. 3 & 4) on new species of plants.

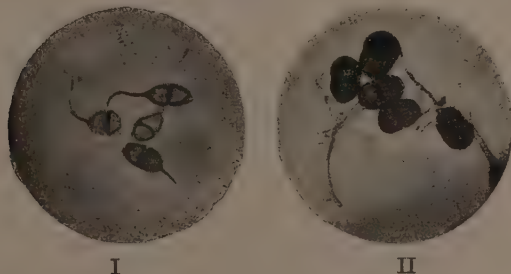
**DESCRIPTION.** 1. *Puccinnia lateritia* Berk. et Curt. On leaves of *Borreria stricta* K. Schum., July — October, 1955, Hazaribagh, Bihar, Leg. H. D. Dubey (Fig. I).

(Previous records - on leaves of *Hedyotis vestita* and *H. auricularia*, Wahjain, Assam (Butler); of *Spermacoce stricta*, Coimbatore, Dehra Dun, and Yelwal, Mysore (Butler); of *S. sp.*, Hoshangabad (Butler); (Butler and Bisby, 1931). Hab. in foliis *Spermacoces* (?) *Cruseae*, *Diodiae*, *Bouvaridiae* (Saccardo, 1899)).

Teleutospore. Sori hypophyllous, rarely on stems, minute, scattered often circinate, roundish, pulverulent, pale brown (scattered sori) to brownish black (circinate sori); spores oblong to ellipsoid, rounded at the lower end, slightly flattened or depressed at the apex and slightly constricted at the septum, chestnut brown,  $25 - 29 \times 17 - 19\mu$ , pedicel hyaline, persistent, longer than the spores.

2. *Puccinia duthiae* Ell. et Tracy. On leaves of *Dicanthium annulatum* Stapf. and *Bothriochloa sp.* (*Amphilopsis odorata*), October - December, 1955, Hazaribagh, Bihar, Leg. H. D. Dubey, (Fig. II).

Figures.



(Previous records - on leaves of *Andropogon pertusus*, Dehra Dun (Duthiae); Poona (Chibber); Ranchi (Mitra); Phulgra (Watt); Dharwar and Kasauli (Butler); of *A. intermedius*, India (Duthiae) - (Butler and Bisby, 1931)).

Uredospores. Sori hypophyllous, scattered, linear, minute (0.5 to 1 mm. long), brown, distinct, pulverulent; spores globose to oval, cinnamon yellow, echinulate,  $22 - 29 \times 17 - 25 \mu$ , with three germ pores; pedicel hyaline, deciduous, a small portion remaining attached with the spores.

Teleutospores. Sori hypophyllous, black, minute, similar to uredia, often developing in the uredial sori; spores ellipsoid to ovoid, rounded at both ends, not thickened above, hardly constricted, smooth, chestnut brown,  $30 - 38 \times 21 - 25 \mu$ , epispore thick; pedicel hyaline, and persistent larger than spore.

3. *Puccinia romagnoliana* Maire et Saccardo. On leaves and stems of *Cyperus iria* Linn., August - October, 1955 Hazaribagh, Bihar, Leg. H. D. Dubey, (Fig. III).

(Previous records - on leaves of *Cyperus rotundus*, Calcutta, Surat, Pusa, Dehra Dun, Saharanpur, Chittagong, Hoshangabad and Samalkota Farm (Butler); Lyallpur (Hafiz Khan); Rangpur (Mitra); Lahore (B. Das); of *C. tegetum*, Pusa (Butler); of *C. compressus* and *C. arenarius*, Chatrapur, Ganjam (Butler); of *C. capitatus*, Surat and *C. tuberosus*, Samalkota (Butler); of *C. sp.*, Nagpur (Pandit); Ranchi (Mitra); Hmawbi, Burma (Butler) - (Butler and Bisby, 1931). Hab. in foliis culmisque Cyperi longi, Liamone et prope Ajaccio in paludosis Corsicae (Saccardo, 1905)).

Uredospores. Sori on leaves and bracts (hypophyllous) and culms, orange, scattered to confluent, minute (0.5 to 1.5 mm.), linear, pulverulent, surrounded by the cleft epidermis; spores globose to ovate, echinulate, pale yellow,  $21 - 24 \times 16 - 20 \mu$ , with two germ pores; pedicel hyaline, deciduous.

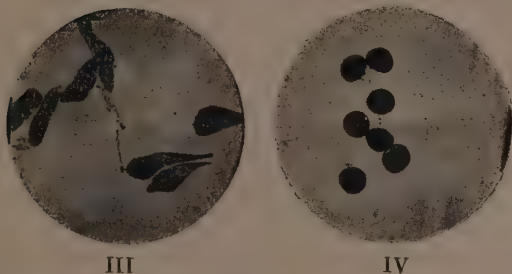
Teleutospores. Sori on leaves and bracts but mostly on culms, minute (1-2 mm.), linear, black, scattered to confluent, non-erumpent; spores clavate to fusoid; apex rounded or conically attenuated, not much thickened, scarcely constricted, tapering downwards, brownish yellow,  $35 - 43 \times 14 - 19 \mu$ , pedicel small, yellow, persistent.

4. *Uromyces mucunae* Rabenh. On leaves of *Mucuna rajada* and *Mucuna cochinchinensis*. September-November, 1955, Hazaribagh, Bihar, Leg. H. D. Dubey (Fig. IV).

(Previous records - on leaves of *Mucuna ? pruriens*, Royal Botanic Garden, Calcutta (Kurz), on *M. (Stizolobium) deeringiana*, Pusa (Butler, Nagpur (Shrivastava); of *M. sp.*, Maymyo and Dehra Dun (Butler); Wyanaad (McRae); Poona (Butler and Bisby, 1931)).



Figures.



Teleutospores. Sori hypophyllous, scattered, minute, roundish, distinct, pulverulent, chocolate brown; spore subglobose, base flattened and conically depressed at the point of pedicel attachment,  $20-21 \times 16-18\mu$  deep brown, epispore minutely echinulate, with a small hyaline popilla at the apex, pedicel long, hyaline, persistent.

The specimens will be deposited in the herbarium of the Indian Agricultural Research Institute, New Delhi.

ACKNOWLEDGEMENTS. My grateful thanks are due to Dr. G. B. Cummins, Purdue University, Indiana, U.S.A., for the authentic identification of the pathogens, and to the Superintendent, Indian Botanic Garden Calcutta, for the identification of host plants. I am also thankful to Mr. V. P. Tewari, Lecturer in Plant Pathology, College of Agriculture, Banaras Hindu University, for his help and co-operation.

Deochand Experiment Station,  
D.V.S., Hazaribagh, Bihar.

#### REFERENCES

- BUTLER, E. J. AND BISBY, G. R. (1931). Scientific Monograph. 1 : 1-237.  
SACCARDO, P. A. (1888). Sylloge fungorum omnium hucusque cognitorum  
7 : 568.
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## DISCOSIA TENZINGII, A NEW SPECIES FROM DARJEELING.

R. C. LACY

(Accepted for publication March 15, 1958)

During a botanical excursion to Darjeeling at the end of October, 1955, an opportunity was availed to visit Tenzing Norgya Sherpa, one of the Everest heroes. After a pleasant interview, plant collection was started. Close to the residence of the Everest hero, at a height of about 6,000 ft., a fungus was collected from several bushes on the leaves of *Osbeckia crinita* Benth. Further collections were obtained at Victoria Falls, also at about 5,000 ft.

*Osbeckia crinita* Benth. is a small bush about 4 ft. high, common in Darjeeling along the hill-sides from 5,000 to 6,000 ft. It belongs to the family Melastomaceae. The entire plant is characteristic in bearing bristly hairs all over the stem, leaves and fruits. The leaves are opposite.

The diseased leaves, when examined, revealed the presence of dimidiate pycnidia, characteristic of Leptostromaceae, approaching Discellaceae in having hysterooid pycnidia at older stages. The presence of 4-5-celled, hyaline, oblong to fusoid conidia, with cilia at each end, place the fungus as a species of *Discosia*. As far as the author has been able to ascertain, the fungus, causing infection on the leaves of *Osbeckia crinita* Benth. is undescribed. It is, therefore, presented here as a new species, named after Tenzing, with his consent, to perpetuate the achievement of this international hero, who conquered Mount Everest on the 29th May 1953.

*Discosia tenzingii* Lacy sp. nov. Infection spots on leaves, 3-5 mm. in diam., circular to angular, separate or confluent, purple to chocolate-brown.

Pycnidia black, epiphyllous, few, separate, dimidiate, circular, flattened,  $54-216 \mu \times 36-108 \mu$ , superficial to innate erumpent, outer wall of pycnidium pseudo-parenchymatous and sterile, inner wall of the lower half hyaline and fertile bearing sporophores. Conidiophores short, unbranched,  $8-10.5 \mu \times 3.5-5 \mu$ , simple; conidia hyaline, crescent to fusoid, 4-5-septate, measuring  $40-60 \mu \times 3.5-4 \mu$ ; contents granular, hyaline, smooth-walled, ciliate at each end; measuring  $64-100 \mu \times 3.5-4 \mu$ , including cilia; parasitic.

On living leaves of *Osbeckia crinita* Benth. (Melastomaceae), 5,000-6,000 ft., Darjeeling, India, 22-10-1955, leg. R.C. Lacy (Type). Type deposited in *Herb. Crypt. Ind. Orient.* New Delhi, India.

*Discosia tenzingii* Lacy spec. nov. Infectionis maculae in foliis, 3-5 mm. diam., circulares vel angulares, disjunctae vel confluentes, purpureae vel atropurpureae.

*Pycnidia nigra*, epiphylla, rara, separata, dimidiata, circularis, complanata, 54 - 216  $\mu$  x 36 - 108  $\mu$ , superficialia vel innato-erumpentia,

## Plate



- Fig. 1. A piece of branch of *Oslechia crinita* Benth., showing infection spots on the dorsal surface of leaves.  $\times \frac{1}{2}$   
 Fig. 2. A portion of leaf spot, dorsal view.  $\times 5$ .  
 Fig. 3. T.s. of infected leaf, passing through a young pycnidium. The presence of sphaeraphides (crystal leaf-tissue is common).  $\times 200$ .  
 Fig. 4. T.s. of infected leaf, passing through an older pycnidium, flattened out.  $\times 200$ .  
 Fig. 5. A portion of the section, magnified.  $\times 475$ .  
 Fig. 6. Presence of hyphae, inter and intra-cellular.  $\times 475$ .  
 Fig. 7. A few conidiophores.  $\times 475$ .  
 Fig. 8. Conidia of different stages and shapes.  $\times 475$ .

parietales pycnidii parietes exteriores pleuriseriati parenchymatici atque steriles, interiores vero hyalini atque fertiles supportantes sporophoros. Conidiophori breves, haud ramosi,  $8-10.5\mu \times 3.5-5\mu$ , simplices; conidia hyalina, falcata vel fusioidea, 4-5-septata, magnit.  $40-60\mu \times 3.5-4\mu$ ; contentis granularibus, hyalinis; parietibus levibus, utroque apice ciliato; ciliis inclusis, magnit.  $64-100\mu \times 3.5-4\mu$

Typus lectus in foliis viventibus *Osbeckiae crinitae* Benth. e familia Melastomacearum, in loco Darjeeling, altit. 5,000 - 6,000 ped. die 22 octobris anni 1955 a R. C. Lacy, et positus in *Herb. Crypt. Ind. Orient.*, New Delhi, India.

#### SUMMARY

A new species of *Discosia*, found at Darjeeling on the leaves of *Osbeckia crinita* Benth. (Melastomaceae) and named after Tenzing as *Discosia tenzingii* Lacy has been described here. This is also the first record of the genus *Discosia* in Darjeeling area.

In conclusion, the author wishes to thank Dr. M. J. Thirumalachar, Chief Mycologist, The Hindusthan Antibiotics, Pimpri, Poona for going through the manuscripts and making helpful suggestions. Thanks are also due to Rev. Fr. Dr. H. Santapau, Chief Botanist, Botanical Survey of India, Calcutta for rendering the Latin diagnosis of the new species.

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## AN UNDESCRIBED SPECIES OF PYRENOCHAETA ON *DOLICHOS BIFLORUS* LINN.

N. N. MOHANTY

(Accepted for publication March 15, 1958)

Kulthi (*Dolichos biflorus* L.) is cultivated quite commonly in Orissa as a pulse and fodder crop. It is generally sown in the high land paddy fields towards the end of September after the harvest of early paddy and harvested by the end of December or early January. A severe leaf spot disease was observed in some of the cultivators' fields in Cuttack District during October - December, 1955. The infected specimens were examined and found to be incited by a new species of fungus belonging to the genus *Pyrenochaeta*. The disease was observed in various intensities in the subsequent years in most of the cultivators' fields of Cuttack, Puri, Balasore and Dhenkanal Districts of the State.

### *Pyrenochaeta dolichi* sp. nov.

Leaf spots pale-brown to almost white in the centre with a dark reddish-brown to almost black border, circular to irregular, 2 to 10 mm. in diameter (fig. 1); sometimes several spots coalescing; on the under surface the spots are reddish-brown in colour with pale-brown to whitish centre, studded with small black pycnidia and conidia of the causal fungus visible to the un-aid eye, Mycelium intercellular, hyaline and septate; conidophores have 2 to 3 hyaline basal cells 12 to 20  $\mu$  x 4 to 6  $\mu$  with light brown, globose, conical shaped or flattened apical cell 8 to 10  $\mu$  in diameter. The dictyosporous conidia are dark brown to black, irregularly globose, 30 to 80  $\mu$  (average 45 to 55  $\mu$ ) in diameter (fig. 2c) Pycnidia yellowish-brown erumpent, reticulate, spherical to flattened, 144 to 174  $\mu$  in diameter (fig. 2a); appendages dark brown, 0 - to 2 - septate, straight to slightly curved, slightly narrower towards the tip, tips rounded, 30 to 120  $\mu$  by 3 to 4.5  $\mu$ . The pynospores are hyaline, spherical, oval to short cylindrical straight to slightly curved 4.5 to 7.5  $\mu$  by 2 to 3 h (fig. 2b); and come out in cirrhi from pycnidia.

On the living leaves of *Dolichos biflours* Linn. Sarichuan, Cuttack, 20-10-1955, N. N. Mohanty; Type deposited in the Herbarium of Mycology and Plant Pathology Section, Bhuneswar and in Herb. Crypt. Ind. Orient, I.A.R.I., New Delhi and Commonwealth Mycological Institute, Kew England.

### *Pyrenochaeta dolichi* spec. nov.

Foliorum maculae pallide brunneae vel fere albae in medio, margine fusce rubro-brunneo vel fere nigro, circulares vel irregulares, 2-10 mm. diam. (fig.1); nonnumquam coalescentes; in pagina inferiore maculae sunt rubro brunnae, centro pallide brunneo vel albedo, consperso pycnidiis nigris atque conidiis, quaevisibilia sunt oculo plano.



Mycelium intercellulare, hyalinum et septatum; conidiophori constate cellulis binis vel ternis basalibus  $12-20\mu \times 4-6\mu$ , cellulis vero apicalibus conicis vel appplanatis, pallide brunneis, globosisque,  $8-10\mu$  diam. conidia dyctyospora fusce brunnea vel nigra, irregulariter globosa,  $30-80\mu$  (mediet:  $45$  to  $55\mu$ ) diam (fig. 2 c). Pycnidia flavo-brunneis, erumpentia, reticulata, sphaerica vel applanata,  $144-174\mu$  diam. (fig. 2 a); appendices fusce brunneae,  $0-2$  - septatae, rectae vel curvatae, paulo angustioribus ad apicem, apicibus rotundis,  $30-120\mu \times 3$  to  $4.5\mu$ . Pycnosporae hyalinae, sphaericae, ovaes vel breviter cylindricae, rectae vel paulum curvatae  $4.5-7.5\mu \times 2-3\mu$  (fig. 2 b), emergentes e pycnidiis in cirros.

Text Fig. 1



Fig. 1. Symptoms of the disease on the leaflets.

In foliis viventibus *Dolichi biflora* Linn. in loco Sarichuan, in provincia Cuttack, die 20 Octobris anni 1955, N. N. Moahanty; Typus positus in herbario Mycologico atque sectione pathologica plantarum ad Bhubaneswar atque in Herb. Crypt. Ind. Orient. I.A.R.I. New Delhi, et in Commonwealth Mycological Instituto, Kew, in Anglia.

The leaf spots occur from seedling to maturity, and first appear as small, reddish-brown lesions. In cases of severe infections, several spots merge together involving a major portion of the leaf blade and the leaflets become chlorotic and usually drop off earlier than the unaffected ones. In such cases, sometimes elliptical brown coloured spots of 3 to 5 mm. in length and 1 to 2 mm. in breadth develop on the stems and brown coloured circular spots of 1 to 3 mm. in diameter develop on the pods.

Text Fig. 2 a - c

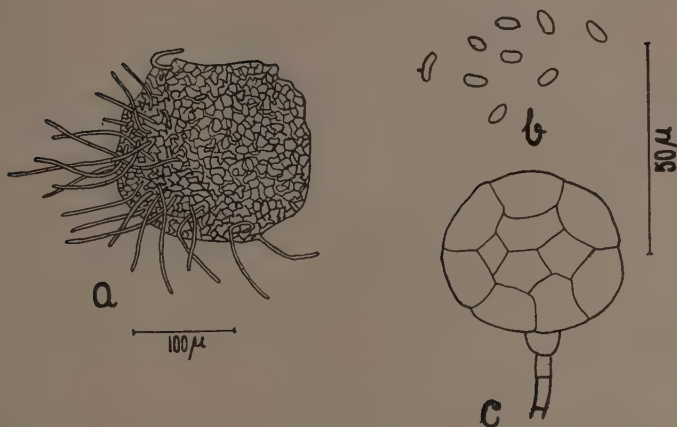


Fig. 2. Camera lucida drawing of (a) Pycnidium, (b) Pycnospores, (c) Conidiophore with conidium.

The fungus causing the disease was brought into culture and the pathogenicity of the fungus was proved.

The conidia of the fungus belonging to the genus *Coniosporium* and the pycnidia of the fungus belonging to genus *Pyrenochaeta* occur on the same leaf spot. Six single conidia and six single pycnospores of the fungus were placed in separate tubes of malt agar. All showed identical growth and produced only globose, dictyosporous dark brown to black conidia measuring 50 to 96  $\mu$  in diameter in culture.

It appears that the two forms i.e. conidial and pycnidial are the different stages of the same fungus and therefore the fungus is proposed to be referred to as *Pyrenochaeta* with a *Coniosporium* stage.

Experiments on the physiological studies of the fungus and control measures for the disease are in progress.

The author is greatly indebted to Mr. Booth of Commonwealth Mycological Institute, and the Director, Commonwealth Mycological Institute, Kew, Surrey, England for having kindly identified the fungus. The author is also grateful to Rev. Father Dr. H. Santapau, Head of the Department of Botany, St. Xavier's College, Bombay for rendering the latin diagnosis.

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Utkal Krushi Mahabidyalaya,  
Bhubaneswar.

## Phytopathological Note

**New records of Hymenomycetes in India.** by B. K. Bakshi:  
At the Forest Research Institute, Dehra Dun, disease survey carried out by the research staff during recent years, revealed the following wood-rotting Hymenomycetes, as new records in India.

### Thelephoraceae

- Coniophora* — *C. cerebella* Pers.  
*Stereum* — *S. ferreum* Berk. & Curt., *S. frustulosum* (Pers.) Fr., *S. rugosum* Pers.  
*Peniophora* — *P. filamentosa* (Berk. & Curt.) Burt., *P. gigantea* (Fr.) Massee and *P. viticola* (Schw.) v. Hohn. & Litsch.

### Polyporaceae

- Fomes* — *F. dependens* (Murr.) Sacc. & Trott., *F. leucophaeus* Mont., *F. ostricoloris* Lloyd, *F. robustus* Karst., *F. roseus* (Alb. & Schw. ex. Fries) Cke., *F. sanfordii* Lloyd, *F. scruposus* (Fr.) Cunn.  
*Inonotus* — *I. nothofagi* Cunn.  
*Lenzites* — *L. palisoti* Fr.  
*Merulius* — *M. aureus* Fr., *M. confluens* Schw., *M. corium* (Pers.) Fr., *M. himantioides* Fr., *M. lacrymans* (Wulf.) Fr., and *M. tremellosus* (Schrad.) Fr.  
*Polyporus* — *P. biformis* Fr., *P. consors* (Berk.) Stevenson, *P. leucospongia* Cke. & Harkness, *P. obtusus* Berk., *P. palustris* Berk. & Curt., *P. tulipiferae* (Schw.) Overh.  
*Poria* — *P. callosa* (Fr.) Cke., *P. corticola* (Fr.) Cke., *P. ferruginosa* (Schrad. ex. Fr.) Karst., *P. nigrescens* Bres., *P. versipora* (Pers.) Rom., *P. xantha* (Fr.) Cke.  
*Trametes* — *T. sepium* Berk.

### Agaricaceae

- Lentinus* — *L. lepideus* Fr.  
*Panellus* — *P. rupicola* (Mass.) Singer

These fungi have been identified or the identities of others confirmed by authorities. Some of the fungi have been described, as they occur in India, from sporophore and culture, while others are awaiting publication. Identities of some of these have also been established by interfertility tests carried out with authenticated species as they occur in Europe and America. Most of the fungi have been recorded from the temperate regions of the Himalayas, whose Hymenomycetes flora resembles largely that of the temperate zones of Europe and N. America.

Forest Research Institute,  
Dehra Dun.

## LETTERS

The American Phytopathological Society this year is celebrating its 50th Anniversary. In connection with this Golden Jubilee Anniversary a series of outstanding symposia by internationally-known scientists is being presented at our meeting with the A.I.B.S. in August 1958. A volume of approximately 1,000 pages containing these 50 symposium papers, as well as contributions by the chairmen of symposia and discussion leaders, will be published by the American Phytopathological Society. Bound in regular book binding the book will be titled: "Plant Pathology—Problems and Progress 1908–1958". Authors include renowned scientists from all over the world.

We feel that this will be the most important publication in the history of Plant Pathology, and will be of interest to research workers, teachers, students, administrators and all interested in agricultural and biological science. Members of your Society would certainly be most interested in the book, and we would appreciate any publicity that could be given to it in your Journal or through your mailing list.

I am enclosing a copy of the program of symposia which shows the fields to be covered and the caliber of the speakers. Additional information is as follows:

**Title of Volume:** "Plant Pathology—Problems and Progress 1908–1958"  
**Description:** Approximately 1,000 page volume, book binding,  
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**Price:** \$8.50  
**Published by:** American Phytopathological Society  
**Order from:** American Phytopathological Society  
P.O. Drawer 1106, New Haven 4, Conn.  
**Publication date:** winter 1958

A limited number of copies will be printed, so orders should be sent in early.

Thank you very much for any publicity that you can give to our publication.

*Very sincerely yours,*

*George A. Zentmyer, Plant Pathologist,  
Chairman, Subcommittee on Publicity  
and Sales of Jubilee Volume*





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Conovor, R.A. (1948).....Studies of two viruses causing mosaic disease of soyabean. *Phytopathology*, 38 : 724-735.

Because of high cost of half-tone blocks carefully made linedrawing on Bristol board in black ink will be preferred. Photographs when necessary should be printed on glossy contrast paper and be of best quality. Full page figures and photographs should be made to reduce  $4+6\frac{1}{2}$  inches, the standard size for all plates. Each author is allowed one page of half-tone illustration for each article or its equivalent and the cost of half-tone blocks and paper in excess will be charged to author. Drawings must be drawn to standard scales, so that they can be compared with one another e.g.,  $\times 10$ ,  $\times 50$ ,  $\times 100$ ,  $\times 250$ ,  $\times 500$  etc. It is not always possible to get magnification at a round figure with a camera lucida but the printer can readily reduce drawings at any magnification to the standard, provided a scale is added to the drawing. The scale should measure from 5 to 10 cm, the longer the better and the printer should be instructed to reduce this line to the desired magnification.

Authors are invited to consult Bisby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), p.p. 38-41 and Riker's 'The preparation of manuscripts for *Phytopathology*, *Phytopathology* 36 : 953-977, 1946, before preparing their mss. and figures.

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